UNIVERSIDADE ESTADUAL DE CAMPINAS

FACULDADE DE ENGENHARIA DE ALIMENTOS

JÚLIO CEZAR JOHNER FLÔRES

# Montagem de um equipamento de extração e fracionamento com fluido supercrítico assistida por prensagem: Adaptações e validação utilizando diferentes matrizes vegetais

# Assembly of an equipment for extraction and fractionation with supercritical fluid assisted by pressing: Adaptations and validation using different vegetable matrices

CAMPINAS

2018

JÚLIO CEZAR JOHNER FLÔRES

# MONTAGEM DE UM EQUIPAMENTO DE EXTRAÇÃO E FRACIONAMENTO COM FLUIDO SUPERCRÍTICO ASSISTIDA POR PRENSAGEM: ADAPTAÇÕES E VALIDAÇÃO UTILIZANDO DIFERENTES MATRIZES VEGETAIS

Tese de doutorado apresentada à Faculdade de Engenharia de Alimentos, da Universidade Estadual de Campinas, como parte dos requisitos exigidos para obtenção do título de Doutor em Engenharia de Alimentos.

Orientadora: Prof.ª Dr.ª MARIA ANGELA DE ALMEIDA MEIRELES PETENATE

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A ata da Defesa, assinada pelos membros da Comissão examinadora, consta no processo de vida acadêmica do aluno.

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# RESUMO

Este trabalho apresenta a reestruturação de uma unidade de escala de laboratório de baixo custo desenvolvida a partir do equipamento de extração de fluido supercrítico montado pelo convênio CNPq/DLR 910016/99-2 (Alemanha). Dentre as etapas desenvolvidas, inclui um levantamento de custos para comparar o valor de uma unidade caseira em relação às unidades comerciais, a validação do equipamento montado e o desenvolvimento de um processo de extração com fluido supercrítico assistida por prensagem. Para validar o extractor, dois separadores anexados e outras partes da unidade, a extração de sementes de urucum e a extração e fracionamento de óleo de funcho foram realizadas utilizando dióxido de carbono supercrítico como solvente. A composição química dos produtos resultantes foi avaliada em termos de abordagem qualitativa utilizando cromatografia em camada delgada. As imagens das placas cromatográficas foram analisadas usando um software gratuito chamado ImageJ, que provou ser uma ferramenta de baixo custo para avaliação do perfil químico dos extratos. Verificou-se que as curvas da extração de urucum estavam de acordo com as obtidas na literatura (3,8% de óleo). Usando o equipamento montado (Denominado SFE-0.1L), os extratos de funcho foram fracionados com sucesso em dois óleos diferentes coletados nos separadores. Os separadores foram validados pelo fracionamento do extrato das sementes de funcho, o óleo essencial foi produzido no segundo separador, e a fração lipídica no primeiro separador. O custo da montagem do equipamento foi inferior a US$ 17,000. Em outra parte desta tese, foram desenvolvidos experimentos de comportamento de fase de alta pressão em extratos fracionados usando um aparelho sintético-visual. Os resultados indicam que os extratos de urucum foram distintos no aspecto visual e no perfil químico. A composição química obtida indica que os extratos de urucum apresentam perfis relevantes de terpenos e fenólicos. Os testes com os extratos revelaram comportamentos de vapor-líquido e vapor-líquido-líquido similares para as composições estudadas. Na última parte desta tese, desenvolveu-se uma nova técnica de extração integrando SFE e prensagem. Esta nova técnica, denominada SFEAP - Extração com Fluido Supercrítico Assistida por Prensagem foi utilizada para extração de polpa de pequi seca e triturada. A técnica SFEAP resultou em uma massa de extrato oito vezes superior ao SFE durante o primeiro minuto de extração, o que correspondeu a um aumento de rendimento de 18,2g/100g de matéria-prima. Para concluir, a unidade SFE-0.1L pode ser efetivamente usada para processos de extração ($CO_2$ supercrítico, etanol, água como solventes), fracionamento e prensagem com ou sem solvente, e os resultados podem ser

usados como referência confiável na construção de unidades SFE em escalas piloto e industriais.

**Palavras-chave:** Pequi, óleo, compostos bioativos, dióxido de carbono.

# ABSTRACT

This work presents the restructuring of a low cost laboratory scale unit developed from the supercritical fluid extraction equipment set forth in the CNPq/DLR 910016/99-2 (Germany) agreement. Among the stages developed, includes a cost survey to compare value of a home unit over that of the commercial units, validation of the assembled equipment and the development of a supercritical fluid extraction process assisted by pressing. To validate the extractor, two attached separators and other parts of the unit, the extraction of annatto seeds and the extraction and fractionation of fennel oil were carried out using supercritical carbon dioxide as solvent. The chemical composition of the resulting products was evaluated in terms of qualitative approach using thin layer chromatography. Images of the chromatographic plates were analyzed using a free software called ImageJ, which proved to be a low cost tool for an evaluation of the chemical profile of the extracts. It was verified that the curves of annatto extraction were in good agreement with those obtained in the literature (3.8% of oil). Using Assembled Equipment (Named SFE-0.1L), the fennel extracts were successfully fractionated into two different oils collected in the separators of the equipment. The separators were validated by fractionation of the fennel seed extract, the essential oil was produced in the second separator, and the lipid fraction in the first separator. The cost of assembling home-made equipment was less than US$ 17,000. Elsewhere in this thesis, high-pressure phase behavior experiments were applied to fractionated extracts using a synthetic-visual apparatus. The results indicate that the annatto extracts were divided in terms of visual aspects and differences in the chemical profile. The chemical composition obtained indicates that the extracts of annatto present relevant profiles of terpenes and phenolics. The tests with the extracts revealed similar vapor-liquid and vapor-liquid-liquid equilibrium phase for the compositions studied. In the last part of this thesis, a new extraction technique was developed by integrating SFE and pressing. This new technique, which is called SFEAP – Supercritical Fluid Extraction Assisted by Pressing, was used for extraction of dried and crushed pequi pulp. The SFEAP technique resulted in an extract mass eight times higher than the SFE during the first minute of extraction, which corresponded to an increase of yield of 18.2g/100g of raw material. To conclude, the SFE-0.1L unit can be effectively used for extraction processes (supercritical $CO_2$, ethanol, water as solvents), fractionation and pressing

with or without solvent, and the results can be used as a reliable reference in the construction of units SFE in pilot and industrial scales.

**Keywords:** Pequi, oil, bioactive compounds, carbon dioxide.

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## *INTRODUÇÃO GERAL, OBJETIVOS E ESTRUTURA DA TESE*

# CAPÍTULO 1

*Introdução Geral, Objetivos e Estrutura da Tese*

## 1.1. INTRODUÇÃO

Os extratos obtidos de matrizes vegetais via extração supercrítica costumam apresentar grande variedade de compostos. Em alguns casos o composto majoritário não é o composto de maior valor comercial. O processo de separação dos extratos tende a facilitar a etapa de comercialização além de agregar valor aos óleos obtidos. O processo de fracionamento dos extratos obtidos via extração supercrítica pode representar uma opção para obter extratos de composição distintas em uma única etapa de extração, apenas controlando os parâmetros de pressão e temperatura diretamente nos separadores. Os compostos presentes nos extratos podem apresentar características de solubilidade distintas no solvente de acordo com a pressão e temperatura utilizadas nos separadores (Brunner, 1994; Martínez & Vance, 2008; Reverchon, 1997).

O extrato de funcho é um exemplo de matéria-prima que é formada por dois grupos de composições bem distintas, sendo um formado pelo óleo vegetal, fração lipídica, e o outro pelo óleo essencial formado principalmente pelos terpenos anetol e fenchona (Coelho et al., 2003; Simandi et al., 1999; Reverchon et al., 1999).

A extração com fluido supercrítico em escala industrial, com extratores maiores que 50 L podendo chegar a até 1000 L ou mais, vêm sendo desenvolvida principalmente em países da Ásia Oriental, Europa e América do Norte onde existem empresas exclusivamente destinadas a dar suporte para a demanda por equipamentos de extração com fluido supercrítico em escala industrial (Products, 2015; Separations, 2015; SEPAREX, 2015; TST, 2015). Na América Latina existem grupos de pesquisas que se dedicam a investigar diferentes matérias-primas e condições de extração utilizando extratores de até doze litros (Meireles, 2003; Moraes et al., 2014). A empresa Bioativos Naturais está incubada na USP e trabalha com dois extratores de dez litros.

No LASEFI (Laboratório de Tecnologia Supercrítica: Extração, Fracionamento e Identificação de Extractos Vegetais) foram construídas várias unidades, um levantamento entre 2009 e 2012 constatou que dos sete equipamentos de extração do laboratório, cinco eram montados no laboratório e outros dois eram unidades comerciais sendo que de todas apenas uma possuía separadores, como pode ser observado na Figura 1.1 de Zabot et al.

(2013). A experiência em construção de unidades dentro do laboratório possibilitou o desenvolvimento de mais um trabalho envolvendo a unidade SFE do Convênio CNPq/DLR (Alemanha), sendo esta voltada tanto à extração como à separação dos extratos. O equipamento original do Convenio (Figura A22 dos anexos) foi projetado e montado na Universidade Técnica de Hamburgo Harburg (TUHH) pelo Prof. Gerd Brunner.

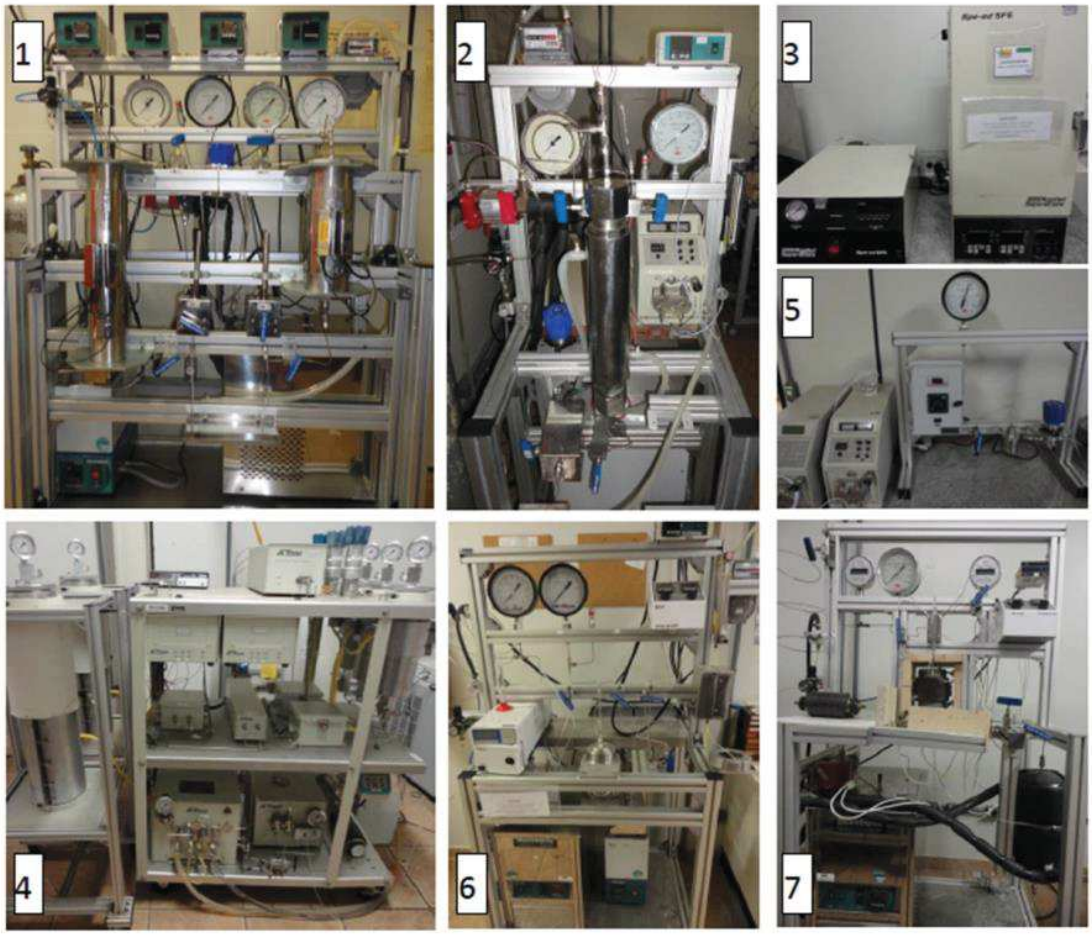


*Figure 1.1 – Equipamentos do LASEFI: (1) SFE-2×1L; (2) SFE-I; (3) SFE-Spe-ed; (4) SFE-2×5L; para micronização: (5) ARADIME; e para hidrólise: (6) HYDRO (Zabot et al., 2013)*

A análise de extratos via TLC (Thin Layer Chromatography) pode ser considerada uma técnica de baixo custo sendo necessárias apenas uma cuba, as placas e uma seringa cromatográfica de 10 µL para ter uma melhor precisão na aplicação das amostras. Juntamente com a utilização de softwares específicos é possível extrair das imagens um sinal que corresponde à intensidade dos pixels das manchas presentes possibilitando uma estimativa da concentração dos compostos (Bernard-Savary & Poole, 2015; Wagner & Blandt, 2001).

Os softwares de tratamento de imagens mais utilizados são aqueles vinculados a um equipamento de fotodocumentação que apresentam elevado custo de aquisição sendo destinados para registro de cromatofolhas e géis de eletroforese. A utilização de softwares

gratuitos apresenta a vantagem de ter livre utilização requerendo apenas uma padronização do sistema de fotodocumentação para que esta etapa não interfira no tratamento das imagens (Harland & Forster, 2012).

## 1.2. JUSTIFICATIVA

O presente trabalho consiste na reestruturação da unidade laboratorial de extração com fluido supercrítico TUHH (Convênio CNPq/DLR Alemanha) e a implementação de uma prensa em seu extrator. Juntamente com a construção e o levantamento do custo foi realizada a validação do equipamento em duas etapas, a validação do extrator e posteriormente a validação dos dois separadores. Muitos trabalhos vêm sendo desenvolvidos com equipamentos comerciais, porém são poucos e desatualizados os dados de custo de montagem deste tipo de equipamento. O baixo custo resultante desta montagem em relação aos equipamentos comerciais produz informações relevantes para os posteriores trabalhos de extração e a aplicação destes resultados em escalas piloto e industrial. Outro aspecto relevante desenvolvido é a validação de um método de baixo custo para a análise dos extratos obtidos via SFE que permitiu identificar a presença de bixina nas amostras além de fornecer uma estimativa de sua concentração com uso do *software* gratuito ImageJ.

## 1.3. OBJETIVOS

### 1.3.1. Objetivo geral

Desenvolvimento de um equipamento de baixo custo para extração e fracionamento de matrizes vegetais.

### 1.3.2. Objetivos específicos

- Construção de um equipamento de extração com fluido supercrítico com custo final de montagem inferior a US$ 16,000.00 usando como base o projeto de uma unidade SFE desenvolvido no âmbito do Convênio CNPq/DLR (Alemanha).

- Montagem de uma unidade de extração com fluido supercrítico utilizando os extratores e os separadores da unidade TUHH (Convênio CNPq/DLR Alemanha), assim como os seus sistemas de aquecimento e refrigeração.

- Validação do equipamento montado utilizando o urucum na etapa de extração e funcho na etapa de separação como matérias-primas modelo.

- Validação de um método de baixo custo para análise dos extratos obtidos que permita quantificar as compostos dos extratos obtidos.

- Montagem de uma prensa no extrator do equipamento SFE-0.1L de forma a possibilitar a utilização do extrator e dos separadores para uma posterior extração com solventes padronizando a pressão que o pistão de prensagem exerce sobre o leito de extração.

## 1.4. ESTRUTURA DO TRABALHO

O texto está apresentado em 7 capítulos. No capitulo **1 – Introdução Geral, Objetivos e Estrutura da Tese** - são apresentados os aspectos mais relevantes para a formulação do trabalho. Neste capítulo são apresentados uma breve introdução do cenário que propiciou o desenvolvimento desta tese, a justificativa, os objetivos delineados e a estrutura planejada para o desenvolvimento dos trabalhos.

No **Capítulo 2 – Revisão Bibliográfica** uma revisão sobre os temas abordados na tese é apresentada. Foram abordados as literaturas sobre os equipamentos e processos de extração e fracionamento com fluido supercrítico e as literaturas que envolvem a polpa do Pequi (*Caryocar brasiliense*).

No **Capítulo 3 – Construção e validação da unidade SFE-0.1L.** Artigo intitulado Construção de um Equipamento de Extração com Fluido Supercrítico: Validação Usando o Urucum e o Funcho e Análise dos Extratos por Cromatografia de Camada Delgada Ligada a Imagem, onde é apresentado o artigo publicado no periódico Food Science and Technology, sua estrutura na forma de fluxograma pode ser observada na Figura 1.2.

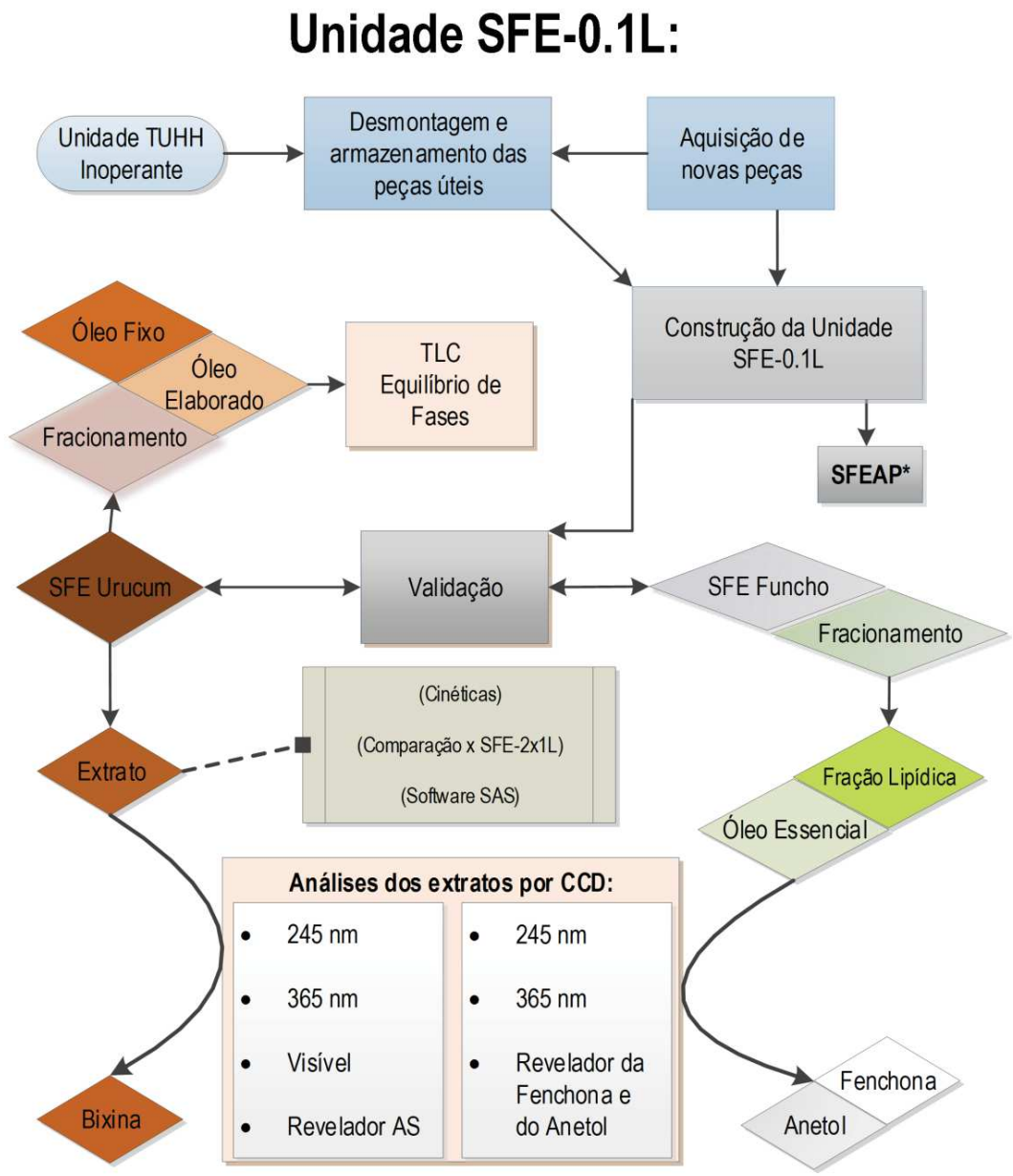


*Figura 1.2 – Diagrama de Blocos do trabalho de montagem do equipamento SFE*

O **Capítulo 4 – Fracionamento via Separadores** é relacionado ao artigo intitulado *Fractionation of Annatto Extracts with Carbon Dioxide Using a Home-Made Equipment*, onde é apresentado o artigo publicado no periódico *Food and Public Health*. Sua estrutura na forma de fluxograma pode ser observada na Figura 1.2.

No **Capítulo 5 – Supercritical fluid extraction assisted by pressing**: A novel extraction technique with promising performance, é apresentado o artigo elaborado para o periódico The Journal of Supercritical Fluids. Sua estrutura na forma de fluxograma pode ser

observada nas Figuras 1.2 e 1.3. Trabalho que compara o processo SFE com o processo SFEAP utilizando a polpa de pequi seca e triturada como matéria-prima.

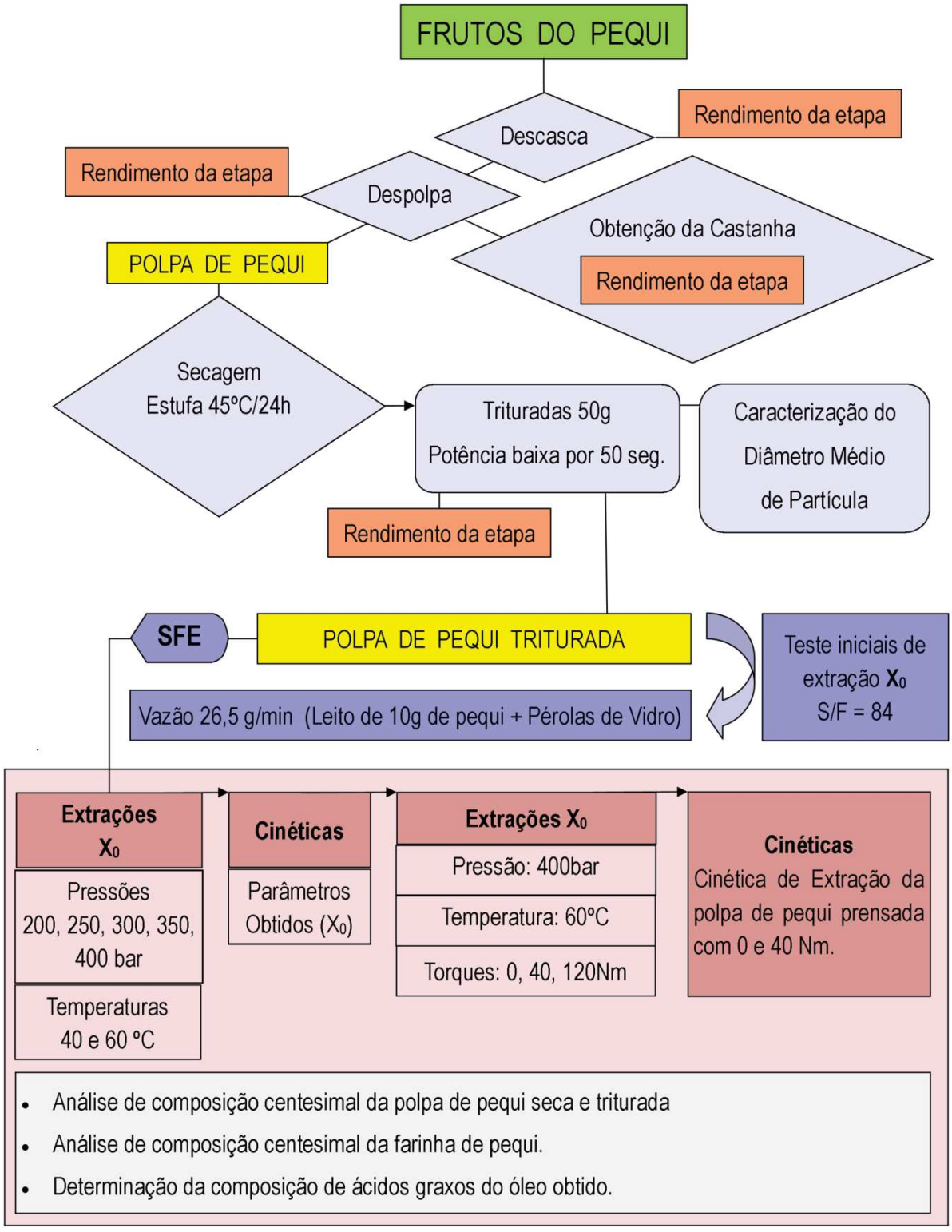


*Figura 1.3 – Diagrama de Blocos do processo SFEAP.*

O **Capítulo 6 – Discussão Geral** traz uma discussão integrada dos resultados obtidos no capítulo 3, 4 e 5. Por fim o capítulo **7 – Conclusões Gerais** apresenta os entendimentos definitivos obtidos com a finalização de todas as etapas.

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# *CAPÍTULO 2*

# ***REVISÃO BIBLIOGRÁFICA***

# CAPÍTULO 2

## 1. FUNDAMENTOS de SFE

A Extração com Fluido Supercrítico (SFE) utiliza um fluido em condições de temperatura e pressão acima do ponto crítico. O processo de remoção de um extrato de uma matriz sólida via SFE começa pela preparação do solvente que será aplicado, passa pela etapa de extração e uma etapa de coleta do extrato por separação. Uma etapa adicional pode ser aplicada para recuperar o solvente utilizado na extração [1].

A tecnologia SFE pode ser realizada em escala laboratorial, piloto ou industrial produzindo no final do processo um extrato livre de solventes. O $CO_2$ é um dos solventes mais empregados devido as suas características, como ser atóxico e por seu ponto crítico (7,38 MPa e 304,2K) ser atingido em baixas temperaturas o que é um fator determinante na preservação de alguns compostos bioativos. No caso da aplicação em extratos vegetais esse solvente acima do ponto crítico apresenta alto poder de solvatação de substâncias com tendências apolares [2].

Um diagrama das etapas comumente usadas em SFE pode ser observado na figura 2.1, onde o extrator é alimentado em seu interior com a matéria sólida e posteriormente o solvente é bombeado no extrator até que sejam atingidas as condições de extração supercríticas do solvente. A mistura de extrato e solvente passa para outro vaso separador onde o extrato é coletado na parte inferior e o solvente na parte superior do vaso podendo ser reutilizado.

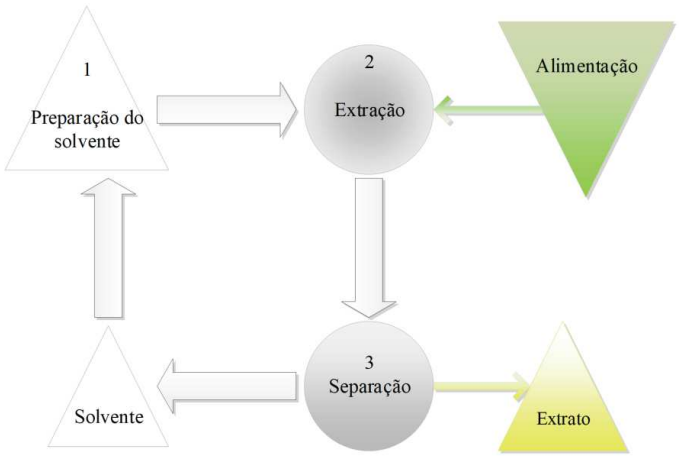


**Figura 2.1 –** Etapas simplificadas do processo SFE.

## 2. MONTAGEM DE EQUIPAMENTOS SFE

No processo SFE apenas um extrator e um separador podem ser utilizados na obtenção do extrato. Para operação em modo semi-contínuo são necessários pelo menos dois extratores, o que evita a necessidade de uma interrupção da extração para o empacotamento com mais matéria-prima dentro do extrator, preparando uma nova extração. Em escala industrial são utilizados até quatro extratores que podem operar de forma semi-contínua com uso de duas bombas no processo [2].

O procedimento de preparo do extrator para uma nova extração pode ser dividido em seis etapas. A primeira etapa é a despressurizarão do extrator, a segunda é a abertura da câmara de extração, posteriormente passa pela descarga de material desengordurado indo para quarta etapa de alimentação com uma nova matriz sólida, na quinta etapa temos o fechamento do extrator e por fim a pressurização do extrator [3].

Na etapa de recuperação do extrato e solvente geralmente são empregados dois separadores, mas podem ser aplicados até quatro separadores em série. A confecção de mais células de pressão eleva o custo final de montagem do equipamento. A principal função de um separador é promover a separação do solvente e extrato podendo operar em condições diferentes buscando o fracionamento do extrato coletado e/ou promover a separação do extrato e solvente mais eficiente. Alguns óleos podem apresentar uma fase muito leve que facilmente pode ser arrastada pelo $CO_2$ mesmo em pressão ambiente. Existe também a possibilidade do fracionamento durante a etapa de extração, sem uso de separadores, neste caso são necessárias pelo menos duas extrações em condições diferentes aumentando assim o tempo de processo quando comparado ao uso das colunas de separação em série depois do extrator.

A extração com $CO_2$ supercrítico aplicada em série com o fracionamento nos separadores é um processo que propicia a obtenção de extratos de alta qualidade devido às baixas temperaturas do processo e a completa eliminação do solvente aplicado; quando na pressão ambiente o $CO_2$ retorna à fase gasosa [4-5].

Um fluxograma mais detalhado do processo SFE em três etapas (Preparação do Solvente, Extração e Recuperação do Extrato) pode ser observado conforme a Figura 2.2.

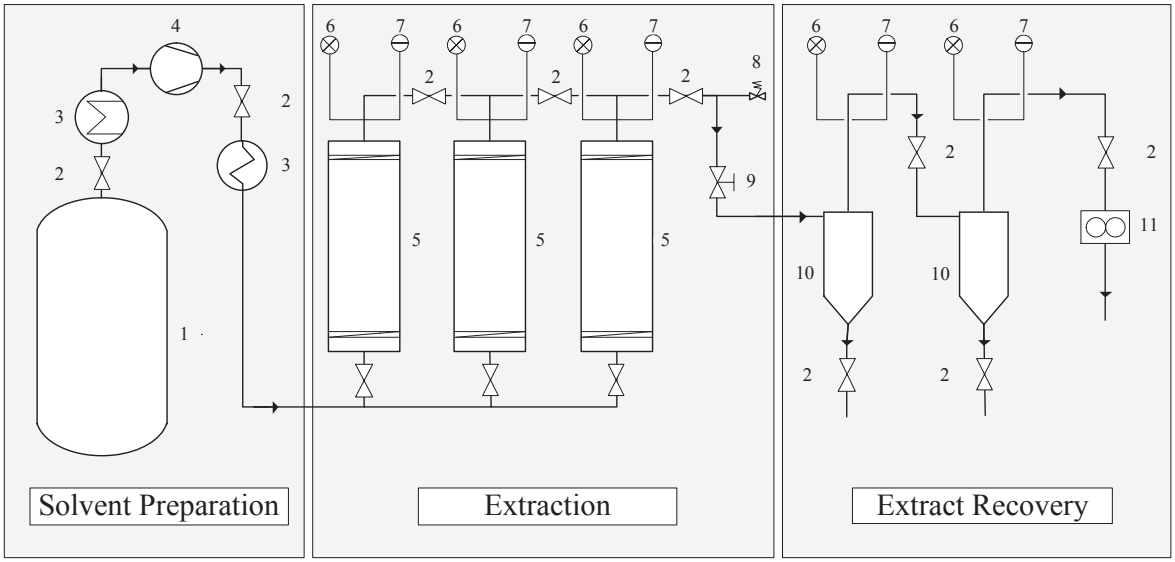


**Figura 2.2** - Diagram of Flow of the Process SFE. (1) $CO_2$ reservoir (2) blocking valves (3) thermostatic bath (4) pump (5) extractors (6) manometers (7) temperature controller (8) safety valve (9) micrometric valve (10) separators (11) flow totalizer.

O componente de uma montagem de unidade SFE mais importante é o extrator, que deve suportar a pressão durante a extração sem apresentar vazamentos e por ser um dos componentes de maior custo do projeto. Devido à utilização de aço inox e a necessidade de uma parede espessa que suporte pressões supercríticas, o extrator é a peça mais pesada de equipamentos na escala piloto e industrial. Suas tampas devem ser manipuladas com cuidado para evitar quedas que podem danificar a rosca e impossibilitar seu fechamento ou deformar as vedações causando vazamentos.

Para montagem da estrutura da unidade que dá suporte ao extrator e aos demais componentes do equipamento em escala piloto ou laboratoriais é indicado o uso de perfilados de alumínio que além de apresentarem alta durabilidade são estruturas de fácil adaptação a qualquer ajuste necessário.

Na fixação das estruturas as cantoneiras e os engates rápidos podem ser utilizados aumentando a rigidez da estrutura, os perfilados de seção quadrada com múltiplos engates facilitam a montagem e futuras adaptações como representado na figura 2.3 e 2.4.

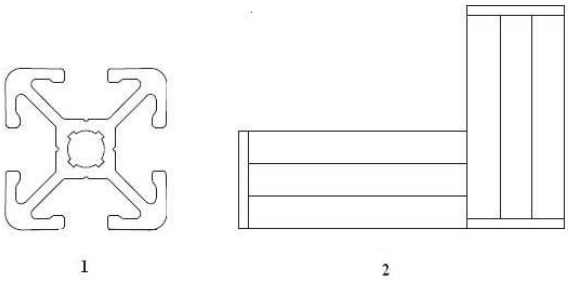


**Figura 2.3** – Perfilado de Alumínio. 1 - Perfil, 2 - Conexão

Várias empresas no mercado trabalham com os perfilados de alumínio e seus acessórios de montagem. Algumas empresas inclusive comercializam as estruturas já montadas de acordo com as características da unidade de extração com fluido supercrítico que se deseja construir.

As dimensões dos perfilados mais comumente usadas para projetos de bancada ou escala piloto são de 30 x 30 mm e ou de 45 x 45 mm. Para construção de unidade em escala industrial são comumente aplicadas plataformas de aço inox que fornecem maior resistência à estrutura que pode possuir mais de um andar e contar com guindastes para possíveis manutenções do equipamento e para movimentar as tampas dos extratores.

Uma chapa de alumínio ou de inox pode ser utilizada como base da estrutura para sustentar os banhos termostáticos como demonstrado na Figura 2.4. A estrutura base do equipamento deve ser montada primeiro e posteriormente devem ser fixados mais perfilados até a estrutura atingir a rigidez necessária.

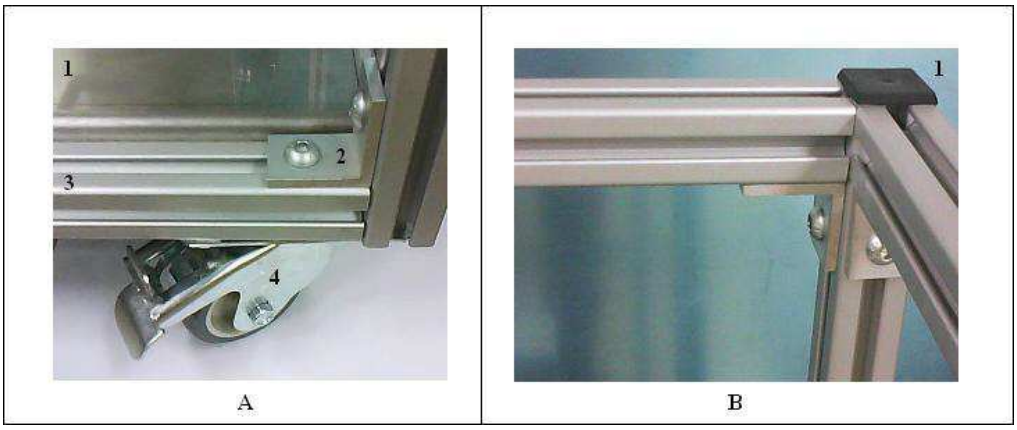


**Figura 2.4** – Estrutura base. Inferior (A) e superior (B): A1 - Base; A2 - Fixação; A3 – Perfilado de alumínio; A4 – Rodízio; B1 – Capa do perfilado.

Com a estrutura base da unidade SFE montada é possível inserir os componentes da linha visando o fácil acesso destes e uma boa visualização dos controladores e indicadores de processo como as válvulas e manômetros.

Os extratores devem ser posicionados de forma a facilitar o acesso para etapa de alimentação do leito com a matriz sólida. Os extratores devem possuir uma camisa térmica que mantenha a temperatura durante o processo de extração. Estas camisas podem ser aquecidas por um banho termostático ou por resistências elétricas. As camisas de aquecimento e refrigeração que promovem a troca térmica pelo bombeamento de um fluido previamente aquecido ou refrigerado preferencialmente devem ser fixadas (soldadas) diretamente na célula de pressão permitindo o contato do fluido com a parede da célula aumentando assim a eficiência da troca térmica. No caso da troca térmica por fluido a distribuição na superfície de contato é mais uniforme do que em relação às resistências elétricas que necessitam de uma manta feita de um bom condutor térmico para distribuir a troca térmica na superfície da célula de pressão. O sistema de troca térmica por resistências elétricas é limitado apenas ao aquecimento do vaso de pressão enquanto a troca por fluido desenvolve tanto o aquecimento quanto a refrigeração, sem mencionar que uma camisa de aquecimento pode estar ligada em série ou paralelo a outras camisas do equipamento. Um único equipamento de aquecimento e bombeamento da água pode ser utilizado para aquecer a serpentina submersa na água por onde passa o $CO_2$ (posicionada antes da entrada do solvente no extrator), a jaqueta de aquecimento do extrator, em serie pode ser ligada aos separadores e ao painel de válvulas.

Ao final da montagem de uma unidade SFE o equipamento apresenta elevado peso, o que dificulta a mobilidade das unidades pilotos e laboratoriais. Os rodízios podem ser inseridos a fim de possibilitar a mobilidade da unidade de extração sendo utilizados pelo menos dois rodízios com freio para que a estrutura possa ser travada.

Tubulações, válvulas e bomba devem suportar trabalhar nas condições de SFE com segurança além do fato de que em toda unidade de extração supercrítica deve ser inserida uma ou mais válvulas de segurança como demonstrado na Figura 2.2-8 para garantir que o sistema opere com um limite máximo de pressão.

Muitos estudos envolvendo a extração com fluido supercrítico têm focado na avaliação econômica do processo. O custo de montagem da unidade é um fator relevante na avaliação de viabilidade econômica de uma planta industrial.

O custo de produção da tecnologia de extração com fluido supercrítico vem direcionando sua aplicação a produtos de alto valor agregado e que não apresentam níveis satisfatórios de extração e ou manutenção das propriedades funcionais quando aplicados aos métodos convencionais de extração [3].

Uma planta de extração por tecnologia SFE não apresenta elevados custo de manutenção. A operação do equipamento é uma das vantagens em relação aos métodos convencionais como destilação e evaporação, porém o investimento inicial é maior [3].

Alguns compostos bioativos presentes nas plantas são de fácil degradação e quando expostos a altas temperaturas podem perder sua atividade funcional. A tecnologia SFE pode ser aplicada como forma de evitar maiores perdas de compostos bioativos devido a trabalhar em baixas temperaturas de extração.

Os compostos bioativos presentes nos extratos vegetais podem apresentar ação antioxidante, anti-inflamatória, antimicrobiana dependendo da matéria-prima de origem. Porém a aplicação de métodos inadequados para a extração destes pode causar a degradação de seus bioativos principalmente com a aplicação de temperaturas elevadas.

Os óleos essenciais são muito voláteis requerendo técnicas que possibilitem sua extração a baixas temperaturas e não apresentem resíduos de solventes. As técnicas tradicionalmente utilizadas são a prensagem mecânica ou a extração com solventes orgânicos que apresentam desvantagens como alto teor residual de óleo na torta prensada no caso da prensagem e resíduos de solventes no caso da extração com solventes orgânicos [5].

O solvente extrator pode ser um fator limitante na avaliação de custos para extração com fluido supercrítico. O $CO_2$ pode ser recuperado após a separação do extrato sendo re-bombeado à linha de extração, mesmo assim ainda existem perdas durante o processo de reciclo.

O solvente apesar de apresentar um custo elevado não é um dos fatores mais relevantes na avaliação de viabilidade. Uma extração utilizando o $CO_2$ com reciclo deste solvente pode apresentar uma perda de 1 a 3% dependendo das condições operacionais a cada ciclo do processo. Para o cálculo de custo do processo pode ser utilizado o valor de 2% de perda de $CO_2$ [1].

## 3. PEQUI

Os consumidores têm buscado cada vez mais produtos com alegações funcionais proveniente de extratos naturais, sejam estes aplicados em alimentos, cosméticos ou fármacos. A indústria farmacêutica e alimentícia, acompanhando esta tendência da redução de formulações sintéticas, tem substituído alguns aditivos por opções mais naturais formadas por extratos com essências que possuam alguma alegação funcional. Dentro deste contexto, os extratos das plantas nativas do Cerrado e da Amazônia brasileiros estão sendo cada vez mais

inseridas no mercado na tentativa de suprir esta demanda por produtos naturais com compostos bioativos.

Com a descoberta das propriedades funcionais de algumas plantas do cerrado brasileiro é crescente número de patentes e artigos científicos envolvendo o tema. Devido às peculiaridades de alguns frutos, sementes, folhas e tubérculos encontrados somente nestas regiões, são necessários processamentos específicos, mudanças de procedimentos ou mesmo adaptações de equipamentos convencionais. Para cada nova matriz vegetal a ter seu potencial econômico estudado são novas possibilidades de aplicações industriais[6-9].

O pequizeiro é um exemplo de planta nativa do cerrado que produz um fruto cuja polpa apresenta grande potencial exploratório devido aos seus compostos funcionais com propriedades antioxidantes, anti-inflamatórias e cicatrizantes[10-12]. A polpa amarelada do pequi possui vitaminas, minerais, compostos fenólicos, óleos essenciais e seu sabor e aroma são marcantes e predominantes quando preparados com outros alimentos[13-17].

A árvore do pequi é uma planta nativa do Brasil, mais especificamente do cerrado brasileiro. O cerrado está presente nos estados de Goiás, Maranhão, Mato Grosso do Sul, Minas Gerais, Tocantins e outros seis estados e é o segundo maior bioma brasileiro [6]. A distribuição do cerrado brasileiro corresponde a 2.036.448 $km^2$ o que representa quase um quarto de todo território. Sua distribuição inclui toda a região central do país, não deve ser confundido com o pequiá fruto da Amazônia cuja planta e fruto apresentam características distintas do fruto do cerrado.

Os frutos do pequi são muito apreciados e representam uma fonte extra de renda às comunidades extrativistas. Com sua popularização vêm sendo estudado e aplicado em plantios comerciais. Seu fruto ainda é pouco explorado em escala industrial.

A polpa do pequi é coletada da planta *Caryocar brasiliense,* seus frutos maduros caem ao solo. O fruto consiste de uma pequena e frágil castanha (Figura 2.5.J) rodeada por um espinhoso e duro envoltório (Figura 2.5.G) que fica coberto pela polpa amarelada (Figura 2.5.F-K), que por sua vez está dentro de um envoltório branco (Figura 2.5.D) coberto por uma fina e resistente casca verde (Figura 2.5.C).

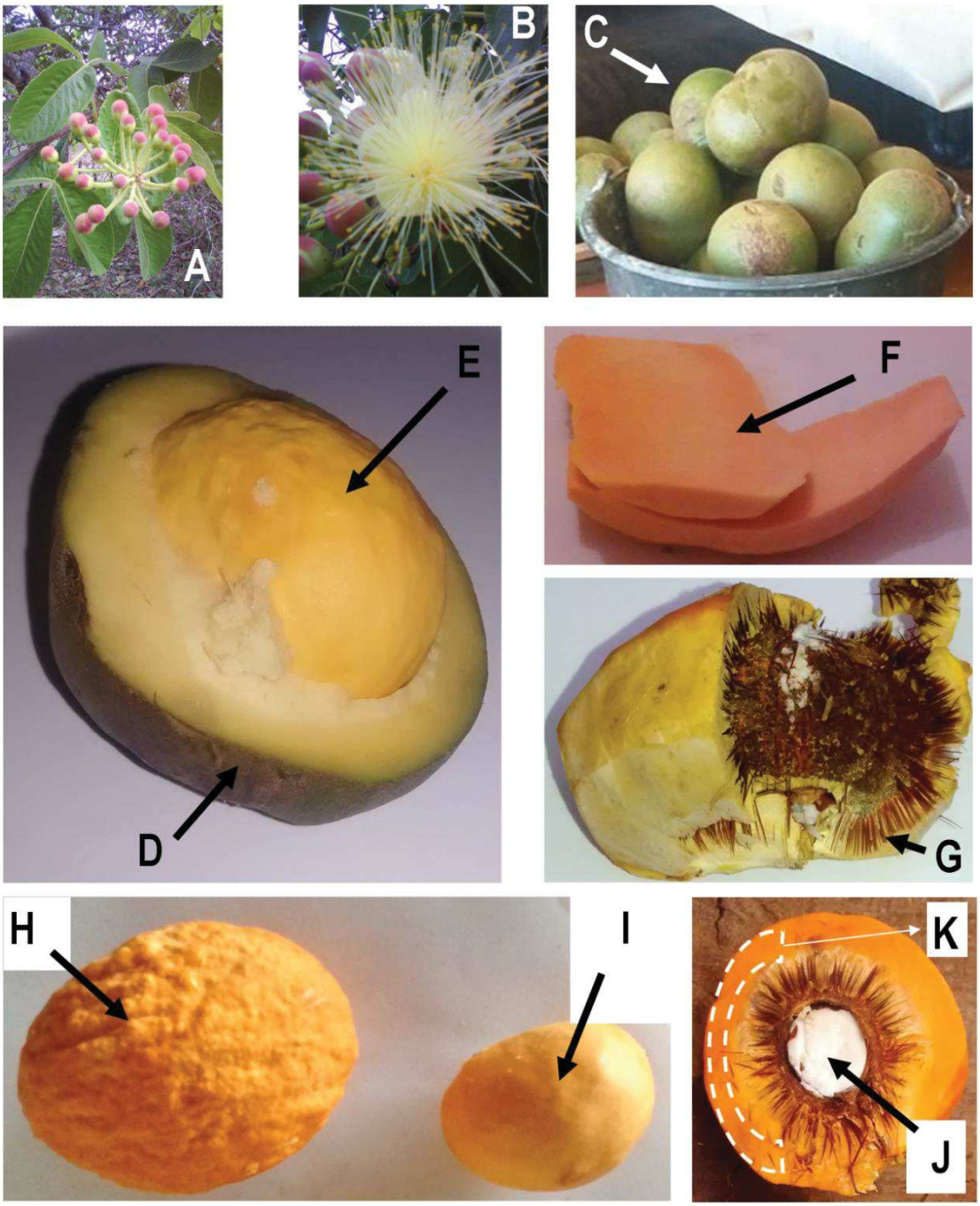


**Figura 2.5 –** Pequi Matogrossense. A - Folhas do pequi, B – Flor do pequizeiro, C – Fruto do pequi, D – Casca do pequi, E – Caroço de pequi, F – Polpa de Pequi cortada em fatias, G – Espinhos presentes no caroço de pequi, H – Caroço do pequi do Mato grosso (Região do Xingu), I – Caroço do Pequi do estado de Goiás, J – Castanha de pequi e K – Região formada pela polpa de pequi.

Uma busca de patentes internacionais utilizando a palavra *Caryocar brasiliense* resultou em 11 registros, sendo seis relacionados à aplicação em cosméticos, dois relacionados a alimentos e três relacionados a outros processos [18-28]. Os registros encontrados no INPI – Instituto Nacional da Propriedade Industrial referentes à palavra pequi

resultaram em 16 registros, sendo quatro relacionados à aplicação em cosméticos, sete em alimentos e cinco em processos diversos [29].

Devido à presença dos espinhos no interior do caroço e a fragilidade da polpa, dentre outras peculiaridades do fruto uma das patentes registradas no INPI [*PI 0800741-1 (28/02/2008)*] é referente a uma máquina despolpadora de pequi [29]. A despolpa manual é demorada e complicada, devido à formação de uma fina camada de óleo em volta dos caroços, que os torna muito escorregadios. Outra possibilidade de processo seria a secagem dos caroços inteiros (polpa, espinhos e castanha) sendo realizada em sequência uma posterior moagem e extração do óleo. Este processo teria como vantagem o aproveitamento do caroço na íntegra e desvantagem a inviabilização da farinha desengordurada para alimentação devido à presença dos espinhos e a mistura do óleo da polpa com o óleo da castanha que não possui carotenoides e apresenta composição majoritária de ácidos graxos saturados.

O óleo de pequi apresenta concentrações de ômega 9 e ômega 3 muito próximas às encontradas no óleo de abacate e no azeite de oliva (Figura 2.6). Este óleo apresenta maiores concentrações de ácido graxo palmítico [30].

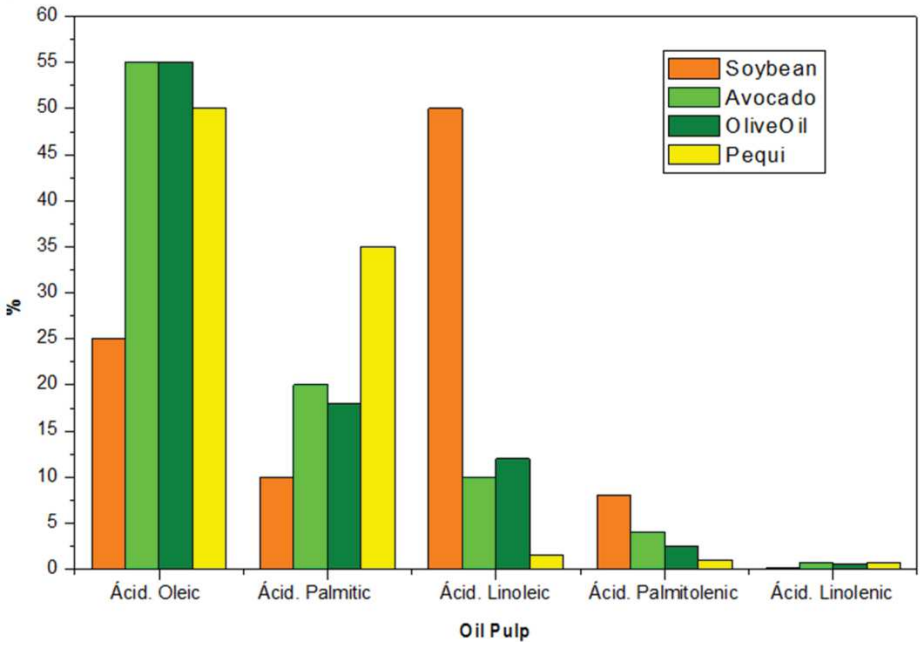


**Figura 2.6 –** Comparação entre os óleos de diferentes matérias-primas [30].

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*CAPÍTULO 3*

# *CONSTRUCTION OF A SUPERCRITICAL FLUID EXTRATION EQUIPMENT*

# CONSTRUCTION OF A SUPERCRITICAL FLUID EXTRACTION (SFE) EQUIPMENT: VALIDATION USING ANNATTO AND FENNEL AND EXTRACT ANALYSIS BY THIN LAYER CHROMATOGRAPHY COUPLED TO IMAGE

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< http://www.scielo.br/scielo.php?pid=S0101-20612016000200210&script=sci_abstract >

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# Construction of a supercritical fluid extraction (SFE) equipment: validation using annatto and fennel and extract analysis by thin layer chromatography coupled to image

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**Abstract**

The present work describes setting up a laboratory unit for supercritical fluid extraction. In addition to its construction, a survey of cost was done to compare the cost of the homemade unit with that of commercial units. The equipment was validated using an extraction of annatto seeds' oil, and the extraction and fractionation of fennel oil were used to validate the two separators; for both systems, the solvent was carbon dioxide. The chemical profiles of annatto and fennel extracts were assessed using thin layer chromatography; the images of the chromatographic plates were processed using the free ImageJ software. The cost survey showed that the homemade equipment has a very low cost (~US$ 16,000) compared to commercial equipment. The extraction curves of annatto were similar to those obtained in the literature (yield of 3.8% oil). The separators were validated, producing both a 2.5% fraction of fennel seed extract rich in essential oils and another extract fraction composed mainly of oleoresins. The ImageJ software proved to be a low-cost tool for obtaining an initial evaluation of the chemical profile of the extracts.

**Keywords:** supercritical fluid extraction; homemade extraction unit; annatto; fennel; thin layer chromatography; ImageJ software.

**Practical Application:** A low-cost (~US$ 16,000) homemade supercritical fluid extraction (SFE) unit for the determination of the process parameters in both steps (extraction and separation) was assembled. A list of the major components and the full details of the construction are provided; using them, the SFE unit can be built by any interested researcher. The bixin present in the annatto extract was detected using thin layer chromatography. Use of the ImageJ software made it possible to evaluate the bixin content in the annatto extracts.

## 1 Introduction

Supercritical fluid technology embodies processes that are performed at pressures and temperatures near or above the critical point of a substance. In the early 1970s, the most popular application was supercritical fluid extraction (SFE), which is often referred to as gas extraction (Brunner, 1994) because of the use of carbon dioxide ($CO_2$) at pressures and temperatures above the critical point (304.2 K and 73.8 bar). Knowledge of the capacity of some condensable gases to solubilize a variety of chemicals is well documented in the literature. For $CO_2$, it has been well documented since the publication of the solubility of several compounds in liquid $CO_2$ by Francis (1954).

Supercritical fluid technology is viable at an industrial scale for several applications. Nonetheless, to date, the most popular application is SFE from solid matrices. The extraction of bioactives from several plants is an attractive field; currently, in Brazil, conventional methods of extraction are practiced at the industrial level. Nonetheless, there is a richness of research being done in the field of SFE by the Brazilian and Latin American scientific communities (Moraes et al., 2014). However, there are only a few semi-industrial units in Brazil and Latin America; these units have extractors of up to 12 liters, i.e., they are of small size compared to the industrial units that are in operation in Asia, North America and Europe. However, experimental data should be used to scale up the process. As discussed by Meireles (2003), small-sized extractors (up to 50 mL) can provide data for selecting the process conditions, such as temperature and pressure. A very careful determination of the extract's chemical composition is required to choose the conditions under which the target compound or a mixture of target compounds is obtained. Then, a very preliminary economic feasibility study should be done. To obtain such data, one can use commercial or homemade SFE units. To the best of our knowledge, the first experimental SFE unit in Brazil was assembled by Meireles & Germer (1992). Since then, several other SFE units have been assembled in LASEFI/DEA/FEA/UNICAMP, as reported in the literature (Ferreira at al., 1993; Pasquel et al., 2000; Santos & Meireles, 2013; Zabot et al., 2014).

The installation of homemade equipment for supercritical extraction with $CO_2$ at a laboratory scale should be a versatile project that is applicable to various raw materials at different extraction conditions, with at least two separators, and should

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allow for adaptations throughout the research to improve functioning (Martínez & Vance, 2008).

Considering that $CO_2$ reservoirs' (laboratory bottles') pressures are between 50 and 60 bar, supercritical extraction equipment at a laboratory scale should contain a thermostatic bath with a coil in its reservoir to cool the $CO_2$ and ensure that it is in the liquid phase during pumping (Brunner, 1994; Zetzl et al., 2003). In this way, the auxiliary pumping system for the chilled fluid, which is usually a solution of ethylene glycol, may be used to cool the last separator and help retain more volatile compounds, thereby guaranteeing a higher purity of the $CO_2$ evaporated at the end of the extraction line and thus facilitating solvent recovery (Reverchon, 1997).

Another component of supercritical extraction equipment that may provide a dual function is the heating bath. It is used to heat the $CO_2$ after pumping, thus keeping it in the supercritical fluid condition, and is used as an auxiliary system to heat the extractor and first separator jackets, as shown in Figure 1 (Zetzl et al., 2003).

Using this strategy, which makes the heating and cooling components multipurpose, the cost of the thermal exchange components in laboratory units may represent only 20% of the total equipment costs. A limiting factor of this strategy is that because the equipment will have only one source for heating and cooling, the temperatures must be set to the closest values of the $CO_2$ pumping conditions and vegetable matrix extraction.

For assembling the various components of the supercritical extraction equipment, 30 × 30 × 6000 mm aluminum profiles, angle bars and screws may be used. These structures are light and adjustable to the needs of the projects, have low acquisition costs and represent only 1.7% of the total installation cost.

Considering the need for supercritical extraction equipment to obtain high-quality extracts without a large initial equipment investment, there was a need for a low-cost SFE unit for use in the development of processes. Then, in 1999, a cooperative project between Brazil (CNPq 910016/99-2) and Germany (Ministry of Research and Education BMBF DLR-IB, BRA 098/78) provided the funds to construct four identical low-cost SFE units at approximately US$ 15,000 each (all taxes included). In Brazil, the following laboratories were involved: LASEFI/DEA/FEA/UNICAMP, LATESC/EQA/CT/UFSC, and LAOS/FEQ/CT/UFPA. In Germany, the laboratory involved was Arbeitsbereich Thermische Verfahrenstechnik (Technical University Hamburg-Harburg (TUHH). The homemade, low-cost units were used extensively in all four laboratories. After 14 years, the SFE unit of LASEFI was remodeled. The purpose of this paper is to make the construction details widely available to the scientific community and to describe the processes that were used to validate the equipment. To validate the equipment, two raw materials for which experimental data are available in literature were used (Albuquerque & Meireles, 2012; Coelho et al., 2003; Moraes et al., 2015; Moura et al., 2005; Reverchon et al., 1999; Simándi et al., 1999). The two materials have different characteristics: (i) annatto (Bixa orellana L.) has the presence of a lipidic fraction that is rich in tocotrienols and a terpenic fraction containing geranilgeraniol, and (ii) sweet fennel (Foeniculum vulgare) contains a lipidic fraction and a terpenic fraction, but in this case, formed by a mixture of terpenes (Moura et al., 2005).

## 2 Materials and methods

The SFE-TUHH was designed and built at the "Thermische Verfahrenstechnik" Laboratory of Professor Gerd Brunner at TUHH – Technische Universitat Hamburg-Harburg – in 2002. The following parts from this equipment were used to assemble the renovated SFE unit, now denoted as SFE-0.1L: the extractor, the separators and part of the heating and cooling systems (Zetzl et al., 2003).

The SFE-0.1L unit was intended to facilitate the control of the pressures on the separators, to modernize process indicators and to develop a new support structure for the equipment components that was less susceptible to corrosion. The original SFE-TUHH design was maintained.

### *2.1 Equipment setup*

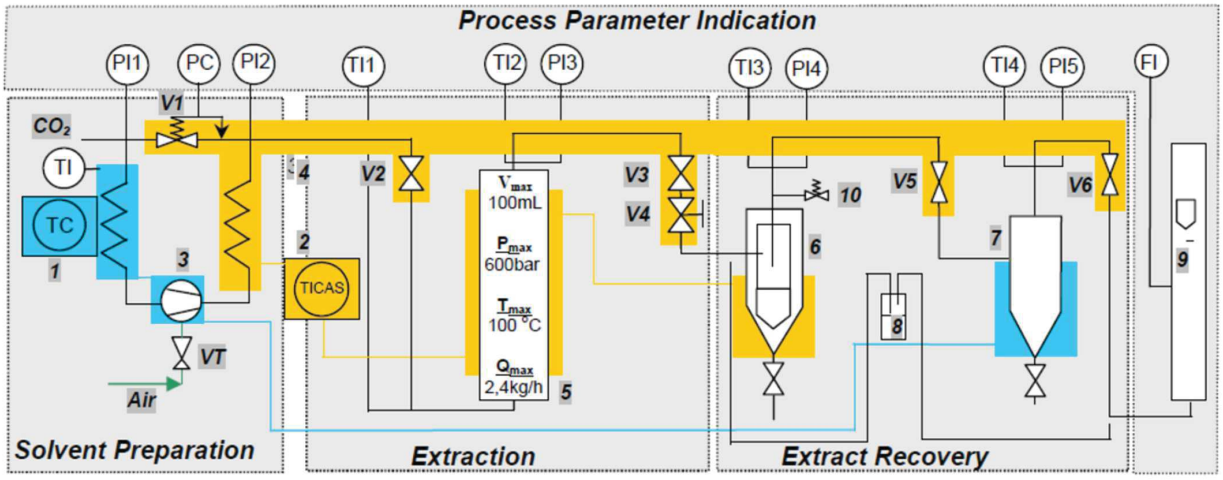
Before starting the renovation of the SFE-TUHH, a list of needed parts was prepared (Table 1). Table 1 lists all of the components used to assemble the SFE-0.1L; the cost of each piece is also presented.

The first step in constructing the SFE-0.1L unit was the assembly of the component support structure using aluminum profiles (30 × 30 × 6000 mm) (SISTEMA, 30 × 30 series, Amparo, Brazil), caps, brackets, screws, bolts and 2 mm stainless steel plates and casters. The main frame of SFE-0.1L contains 4 pieces of 800 mm, 5 pieces of 710 mm and 7 pieces of 510 mm These pieces units were attached to the primary structure (Figures 2-4). The two steel plates were assembled based on the dimensions of the previously described structure profiles; that is, (770 × 570 × 2 mm) and (300 × 420 × 2 mm). Subsequently, all of the components were located with the objective of easy access to the extractor, separators, valve panel and process indicators. The 1/8-inch stainless steel tubing (Fopil, ASTMA269S, Campinas, Brazil) was folded and reinserted into the structure by connecting the points, in accordance with the flow diagram in Figure 5.

Following the installation of the tubing using the connections and components in Figure 5, thermal insulation was installed. The silicon hoses and their insulation were connected to the heating and cooling components.

The valve box was constructed of stainless steel to avoid corrosion from constant contact with the heating fluid, with 22-mm connections to facilitate fluid output flow. The insulation, similar to all of the heating and cooling components, was made using a 3-mm-thick elastomeric blanket (Epex, Vidoflex M2, Blumenau, Brazil).

In building the unit, a system was installed to reduce pressure variations in the extractor. Pressure variation during the extraction process may cause solute and solvent waste (Martínez & Vance, 2008). Pneumatic pumps may cause pressure oscillations by their own variation in supplying compressed air to the compressor. An efficient system consists of a backpressure valve installed with its inlet after the heating of the $CO_2$ and the outlet installed before the cooling coil inlet (Zetzl et al., 2003).




| | |
|---|---|
| 1 | Cooler (Condenser). $CO_2$ cooling loop |
| 2 | Heater unit |
| 3 | Air driven $CO_2$ Pump ("Booster") 1:110 |
| 4 | Water-heated box for expansion valves |
| 5 | Extraction Autoclave, 100 mL, heating jacket |
| 6 | Cyclone with inner parts, heating jacket |
| 7 | Separator with cooling jacket |
| 8 | Alternative sampling line in glass tubes |
| 9 | Flow Meter (kg $CO_2$/h) at NPT |
| 10 | Safety valve for cyclone and separator |

| | |
|---|---|
| TI 1 | Flow Temperature at Autoclave Inlet |
| TI 2 | Flow Temperature at Autoclave Outlet |
| TI 3 | Flow Temperature at Cyclone Outlet |
| TI 4 | Flow Temperature at Separator Outlet |

| | |
|---|---|
| VT | Air Throttle - Booster Piston Frequency |
| V1 | Back Pressure Regulator - Process Pressure |
| V2 | Inlet I/O Valve for Extraction Autoclave |
| V3 | Outlet I/O Valve for Extraction Autoclave |
| V4 | Micro Needle Valve - Fluid Flow Rate |
| V5 | Regulation Valve - Cyclone Pressure |
| V6 | Regulation Valve - Separator Pressure |

| | |
|---|---|
| PI 1 | Pressure $CO_2$ Supply |
| PI 2 | Pressure $CO_2$ Solvent Preparation Cycle |
| PI 3 | Pressure Extraction Autoclave Outlet |
| PI 4 | Pressure Cyclone |
| PI 5 | Pressure Separator |

**Figure 1**. Flow diagram for the Technical University Hamburg Harburg (TUHH) experimental apparatus (Zetzl et al., 2003). The unit assembled in TUHH and send to LASEFI was denoted as SFE-TUHH.

In this way, when the system is over pressure, the pressure valve adjusts, with recycling keeping constant pressure in the extractor. The Maximator M 111 Series pump used to install the SFE-0.1L unit was fixed as close to the thermostatic bath as possible.

Pressurization tests were done on the extraction cells after the equipment was installed. The pressure cells were filled with glass beads and subjected to 350-bar pressure for a static time of 20 minutes, followed by a flow period with solvent flow rate of 15 g/min for 30 minutes. This was done to test the pump's functioning and to evaluate possible oscillations in the pressure and temperature during the process (Appendix A).

The positioning of the components in the unit prioritized the functionality of the equipment in such a way that all process indicators were visible, easily managed and in front of the operator (Appendix B).

The control and process valves were positioned on the valve panel, with the micrometering valve being responsible for regulating the flow rate (Appendix C). To operate the unit without the separators, auxiliary tubing was developed that connected

**Table 1**. SFE-0.1L Estimated Cost and Components.

| Product Name | Qty | Price | Total (USD) |
|---|---|---|---|
| Extractor 100 mL* | 1 | 748.40 | 748.40 |
| Separator 90 mL* | 2 | 916.80 | 1,833.61 |
| Jacket* | 3 | 100.03 | 300.09 |
| Valve box (Maq'nagua, Serra Negra, Brazil) | 1 | 157.23 | 157.23 |
| Aluminum plate ($m^2$) (Autic, Campinas, Brazil) | 3 | 30.50 | 91.51 |
| Aluminum profile 6 m (Sistema, 30 × 30 series, Amparo, Brazil) | 8.5 | 11.32 | 96.23 |
| Angle (Sistema, 30 × 30 series, Amparo, Brazil) | 40 | 1.57 | 62.89 |
| Square nut and screws (Sistema, 30 × 30 series, Amparo, Brazil) | 150 | 0.68 | 101.42 |
| End cap (Sistema, 30 × 30 series, Amparo, Brazil) | 8 | 0.79 | 6.29 |
| Swivel caster (Colson, 3", Araucária, Brazil) | 4 | 6.92 | 27.67 |
| Manometer* (WIKA, EN8371-1, Klingenberg, Alemanha) | 5 | 341.19 | 1,705.97 |
| Safety valve (Swagelok, SS-4R3AS, São Paulo, Brazil) | 1 | 237.23 | 237.23 |
| Temperature indicator (Pyrotec, T4WM-TP100, Campinas, Brazil) | 1 | 190.25 | 190.25 |
| Termocouple (Pyrotec, TP100, Campinas, Brazil) | 5 | 20.35 | 101.73 |
| Flowmeter (Cole Parmer, PMR1, IL, USA) | 1 | 336.48 | 336.48 |
| Flow totalizer (Itrón, G25, Americana, Brazil) | 1 | 40.88 | 40.88 |
| Tubing 1/8" (6 m) (Fopil, ASTMA269S, Campinas, Brazil) | 2 | 89.62 | 179.25 |
| Ferrule 1/8" (Fopil, Campinas, Brazil) | 10 | 3.24 | 32.42 |
| Connector Tee OD 1/8" (Fopil, ASTMA276, Campinas, Brazil) | 8 | 44.30 | 354.39 |
| Connector Tee side NPT 1/4" (Fopil, A.276316, Campinas, Brazil) | 4 | 20.13 | 80.50 |
| Connector OD-OD (Fopil, ASTMA269TP316S, Campinas, Brazil) | 3 | 20.12 | 60.35 |
| Connector OD-NPT (Fopil, ASTMA269TP316S, Campinas, Brazil) | 2 | 11.80 | 23.60 |
| $CO_2$ filter (Swagelok, F series, São Paulo, Brazil) | 1 | 96.38 | 96.38 |
| Blocking valve (Autoclave Engineers, 10V2071, PA, USA) | 5 | 165.17 | 825.83 |
| Micrometering valve (Autoclave Engineers, 10VRM2812, PA, USA) | 1 | 466.53 | 466.53 |
| Back pressure valve (Tescon, 44-1800, Sorocaba, Brazil) | 1 | 366.04 | 366.04 |
| Back pressure valve (Tescon, 44-2200, Sorocaba, Brazil) | 1 | 602.91 | 602.91 |
| Back pressure valve (Tescon, 26-1700, Sorocaba, Brazil) | 1 | 1,550.33 | 1,550.33 |
| Air pressure regulator (Norgren, R07-100-RNKA, São Paulo, Brazil) | 1 | 100.94 | 100.94 |
| Air-driven $CO_2$ pump (Maximator, M-111L, Nordhausen, Germany) | 1 | 1,861.64 | 1,861.64 |
| Heating bath (Thermo Haake, DC30/DL30, Eindhoven, Holanda) | 1 | 1,532.84 | 1,532.84 |
| Cooling bath (Thermo Haake, C10, Eindhoven, Holanda) | 1 | 1,691.82 | 1,691.82 |
| Ball valve (Japi, ball, Jundiaí, Brazil) | 2 | 7.36 | 14.72 |
| Hose npt adapter (Amanco, Campinas, Brazil) | 2 | 3.46 | 6.92 |
| Hose connector 90 elbow (Elgo, 1203, Itu, Brazil) | 1 | 1.73 | 1.73 |
| Insulation tube Ø - 1/8" (m) (Armaflex, AFM-10, Campinas, Brazil) | 1 | 1.83 | 1.83 |
| Insulation sheets (Epex, Vidoflex M2, Blumenau, Brazil) | 0.6 | 24.45 | 14.67 |
| Insulation tube Ø - 22 mm (Isolan, Campinas, Brazil) | 5 | 0.94 | 4.72 |
| Plastic tee conector (Amanco, Campinas, Brazil) | 4 | 1.96 | 7.83 |
| Silicone hose (5 m) (Sinergia, 200 × 200, Campinas, Brazil) | 1 | 22.17 | 22.17 |
| | | Total | 15,938.24 |

*Calculated Cost.

the output of the micrometering valve with the external part of the valve panel, thus allowing the extracts to be collected immediately after extraction (Appendix D).

### *2.2 Equipment validation*

To validate the equipment, two raw materials, annatto and sweet fennel, were used. The annatto was used to validate the extraction line without the use of the separators, and the fennel was used to validate the extraction followed by separation.

### *2.3 Raw materials preparation*

The annatto seeds were acquired at the Estação do Grão Ltda. (São Paulo, Brazil) and stored at 255 K. They were packed into the extractor without any further preparation, as indicated by Silva et al. (2008), Albuquerque & Meireles (2012) and Moraes et al. (2015).

The fennel seeds were acquired in the municipal market of Campinas, Brazil at "Temperos Brasil" and kept at 255 K. The fennel seed preparation procedure was adapted from the

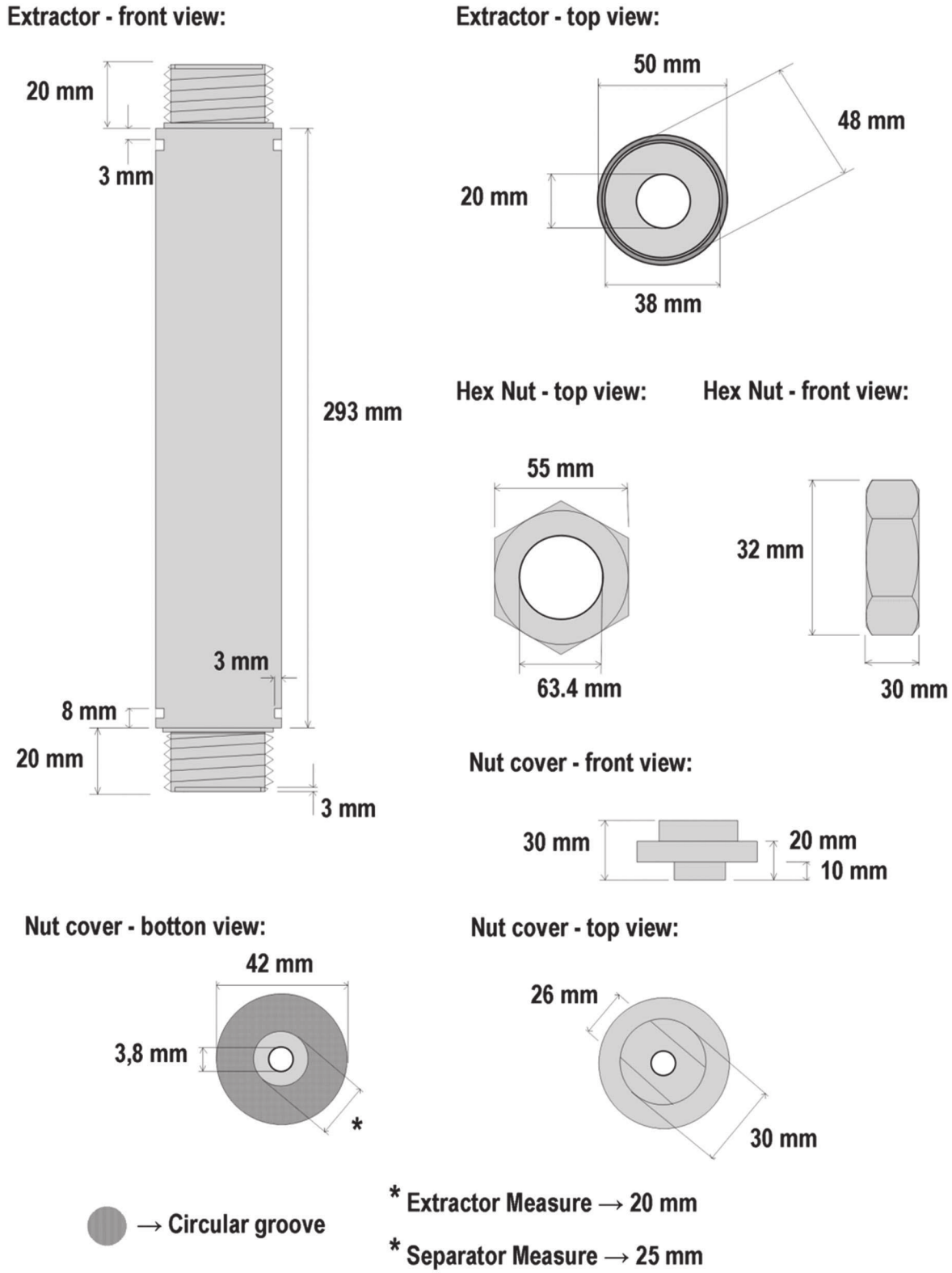
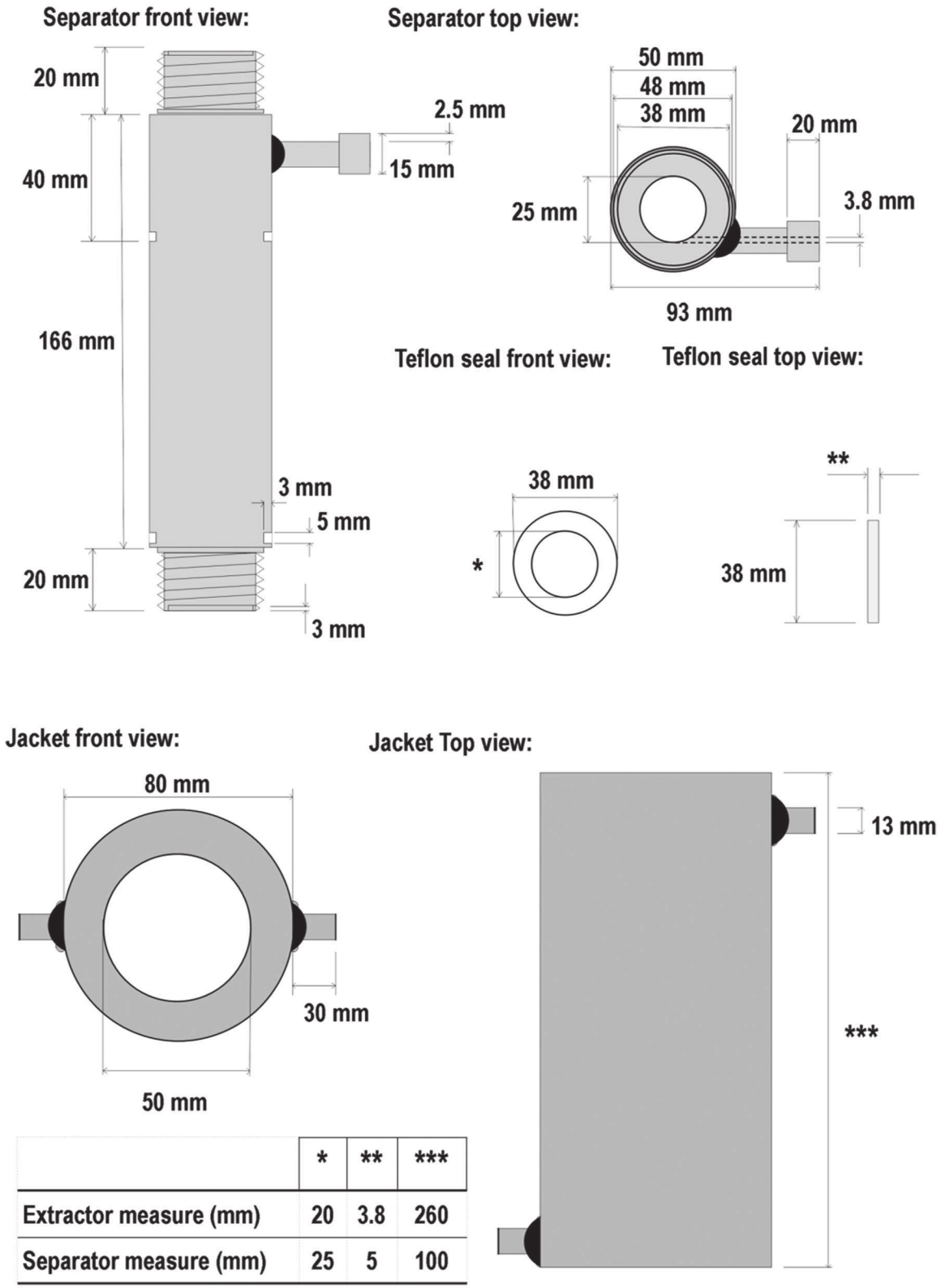



**Figure 2**. SFE-0.1L extractor and nuts.




| | * | ** | *** |
|---|---|---|---|
| Extractor measure (mm) | 20 | 3.8 | 260 |
| Separator measure (mm) | 25 | 5 | 100 |

**Figure 3**. SFE-0.1L separators, teflon seal and jacket.

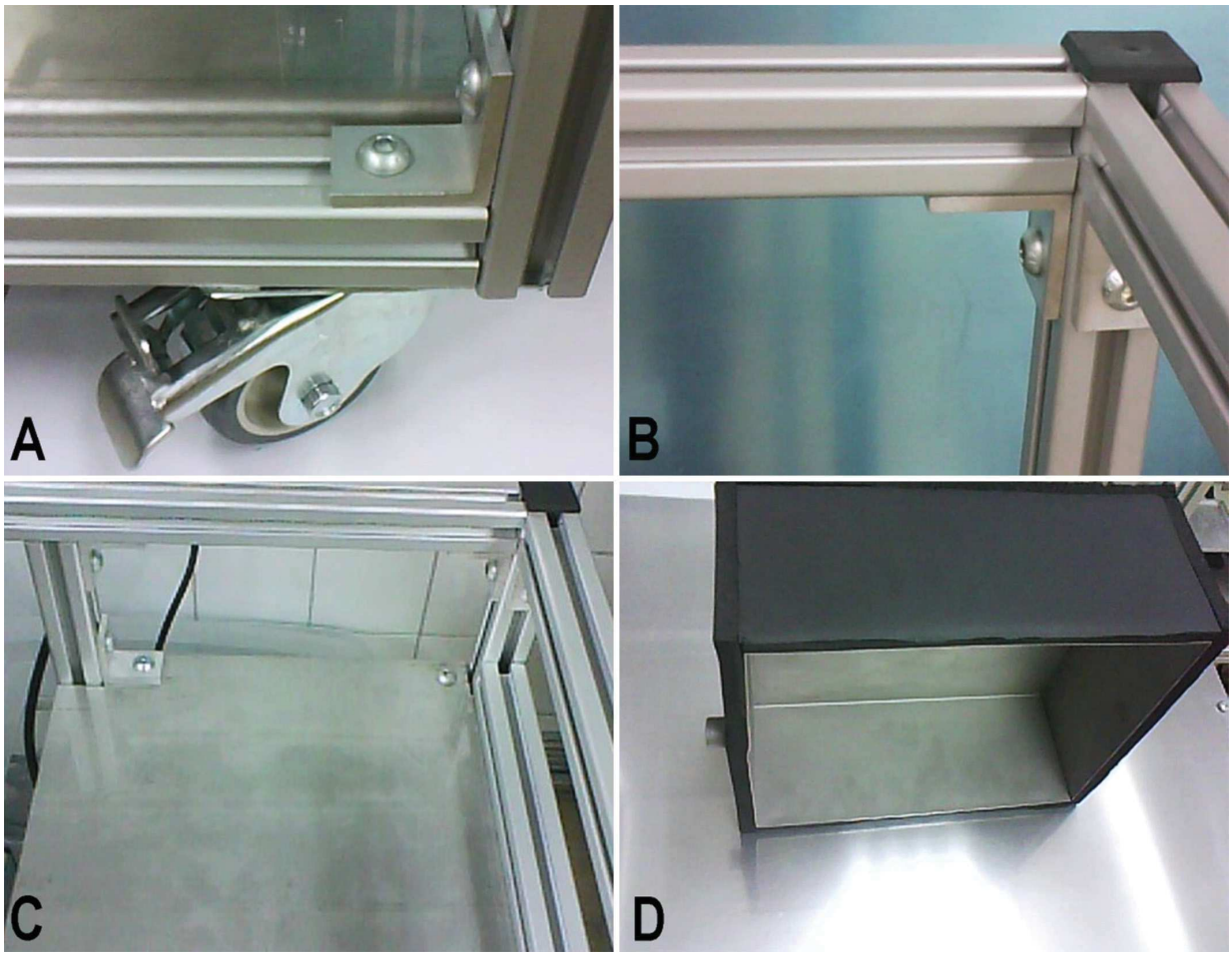



**Figure 4**. Photos of the structure: (A) caster; (B) aluminum profile; (C) valve box base; (D) valve box.

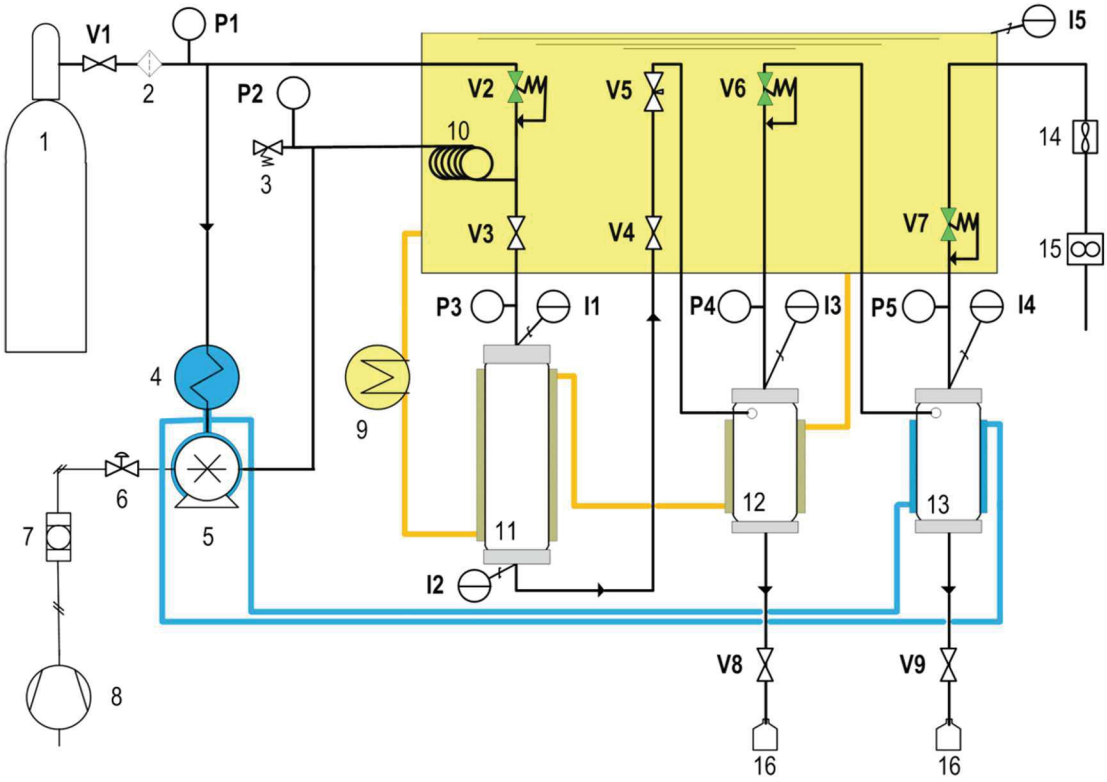
work of Moura et al. (2005), with the frozen seeds being ground in a mill (Marconi, model 340, São Paulo, Brazil) using a 3-mm sieve into 20-g batches. To characterize the average particulate diameter by the standard method of ASABE (American Society of Agricultural and Biological Engineers, 2008), an electromagnetic agitator was used (Bertel, model 602, São Paulo, Brazil) with screens of the Tyler mesh series (16, 24, 32, 48 and 80 mesh). The prepared seeds were then packed into the extractor.

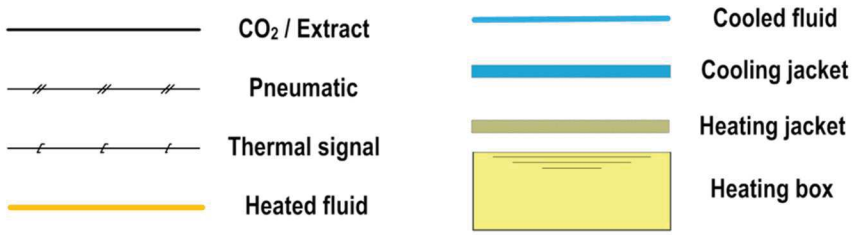
### *2.4 Extraction and separation*

The extractor of the SFE-0.1L unit has a volume of 100 mL and an internal height/diameter relationship of 19 ($H_B/D_B = 19$). The two separators have a 90 mL volume, with a tangential entrance equipped with thermocouples that reach half the height of the cylinder, allowing estimates of the wall boundary conditions and of the temperatures at the central points of the cylinders.

For annatto and fennel, the solvent used was carbon dioxide (99% purity, White Martins, Brazil); the bath was set at 268 K to assure $CO_2$ in the liquid phase at the pump entrance. The extractor pressure was 200 bar, and the temperature was 313 K according to the procedure used by Moraes et al. (2015), with a flow rate of 23.7 g/min and 60 g feeding into the extractor. The extracts were collected immediately after the micrometering valve using glass flasks that were also connected to the flowmeter (Cole Parmer, PMR1, IL, USA) and then to the flow totalizer (Itrón, G25, Americana, Brazil). The ratio of the mass of solvent to the feed mass (S/F) was comparable to the extraction curve of the SFE-0.1L unit for the annatto extraction data of the SFE-2×1L unit, built and validated at LASEFI/DEA/FEA/UNICAMP (Zabot et al., 2014).

The extract masses were weighed, and the extraction yield was calculated as the mass of extract divided by the mass of feed (dry basis). The experimental overall extraction curve (OEC), that is, the cumulated mass of extract versus the extraction time, can be interpreted as containing three stages: (i) the constant extraction rate period (CER), where convection is the major mass transfer mechanism; (ii) the falling extraction rate period (FER), which is dominated by convection and diffusion; and the last stage, (iii) the diffusion-controlled period (DC), where the




| List of equipments | | | | List of valves | |
|---|---|---|---|---|---|
| 1 | $CO_2$ reservoir | 11 | Extractor | V(1,3,4) | Blocking valve |
| 2 | $CO_2$ filter | 12,13 | Separator | V(8,9) | Blocking valve |
| 4 | Cooling bath | 16 | Extrac collecting vessel | V2 | Back pressure (600 bar) |
| 5 | Air-driven $CO_2$ pump | List of instruments | | V6 | Back pressure (400 bar) |
| 7 | Air filter | 14 | Flowmeter | V7 | Back pressure (100 bar) |
| 8 | Air compressor | 15 | Flow totalizer | V5 | Micrometering valve |
| 9 | Heating bath | I(1,2,3,4,5) - Temperature Indicator | | 3 | Safety valve |
| 10 | Serpentine tube | P(1,2,3,4,5) - Pressure gauge | | 6 | Control (air flow) |

**Figure 5**. Flow diagram for the SFE-0.1L experimental apparatus.

diffusion of solute from the solid structures defines the mass transfer rate (Meireles, 2008). The overall extraction curve (OEC) was adjusted to a spline model containing three straight lines using the SAS 9.2® software according to the steps described by Meireles (2008) and Zabot et al. (2014).

Fennel extract was obtained at 200 bar and 313 K according to the procedure used by Reverchon et al. (1999), with a flow rate of 12 g/min and S/F of 10. The conditions in separators 1 (80 bar, 312 K) and 2 (20 bar, 279 K) were adapted from the works of Coelho et al. (2003) and Simándi et al. (1999). After 60 minutes of extraction, the extracts were stored at 255 K for later analyses.

### *2.5 Analyses of extracts*

Annatto extracts: Extracts were analyzed using Thin Layer Chromatography (TLC). A bixin standard was prepared according to the procedure performed by Albuquerque & Meireles (2012), using a Shaker Incubator (Marconi, MA420, São Paulo, Brazil) in which annatto seeds were exhaustively extracted with acetone for 6 h (200 rpm, 298 K). TLC plates (Macherey-Nagel DC-Fertigfolien Alugram®, XTSILG, Darmstadt, Germany) were used; the mobile phase was hexane:ethyl acetate:formic acid (79:18:3), as optimized by Albuquerque (2013). For fennel extracts, the TLC plates (Macherey-Nagel DC-Fertigfolien Alugram®, XTSILG/$UV_{254}$, Darmstadt, Germany) were used; the mobile phase was toluene:ethyl acetate (6:4), as described by Wagner & Bladt (2001).

The pattern of bixin (3 µL) was applied 3 times on the chromatographic plate to test the repeatability of the method, and 6 points of this pattern with different masses of bixin (0.6, 1.2, 2.4, 4.8, 9.6 and 19.2 µg) were applied on the same plate to evaluate the linearity of the developed method.

The pattern of bixin (3 µL) was inserted at the first point of the plate, followed by the extracts of annatto that were collected at selected extraction times. The extracts (1 µL) were inserted directly onto the plates based on the increasing sequence of extraction times. The plates were photographed with and without the developer AS - anisaldehyde sulfuric acid reagent – and heated for 5 min at 383 K using a tank equipped with a UV lamp: Short Wave (254 nm) - Long Wave (366 nm) – (Entela, UVGL-58, USA).

The fennel extracts obtained from the first and second separator were diluted with toluene in the proportion extract:toluene (1:1); 1 µL of the extract was used (extract in separator-1) with 3 repeated applications of 1 µL for the extract from separator-2. The eluted plates were photographed in a tank equipped with a UV light (254 and 366 nm). The plates were heated for 5 min at 383 K before and after spraying with concentrated sulfuric acid developer.

The images of the chromatographic plates were processed using the free ImageJ software (Ferreira & Rasband, 2010). A location with adequate illumination for the possible points was prepared first, so the areas of the plate with less illumination would not influence the images. A standard photodocumentation height, the point where the plate was placed and the point where the machine would be positioned to take photos, was selected. The camera was fixed using a support consisting of a stand with handles, with the lens and the TLC plate remaining parallel and at a distance of 30 cm. Subsequently, the photos were opened in ImageJ by the command <File-Open>. A plate without any extract was tested to evaluate the efficiency of the photodocumentation system, aiming for an image with a standard open surface plane using the Interactive 3D Surface Plot tool.

ImageJ was used to calculate the "Ratio to Front" ($R_f$) of the plates, first testing to find the best way to visualize the bands on the plate. By selecting the command <Edit-Invert> to invert the colors of the plate and comparing the result with the original colors of the image, this tool may provide a more accurate measurement, even for compounds that are present at low concentrations or for those with coloration bands close to the intensity of the pixels on the bottom of the plate.

The best image for measuring the $R_f$ of each compound was determined by the commands <*Straight*, segmented of freehand lines - Analyze - Measure (Results) - Analyze - Label>, which were repeated until all necessary measurements were obtained. During the measurement, the lines were traced without deviating from the slope of the straight line; that information was provided along with the results of the measurements.

The areas of the peaks were calculated to estimate the effect of different bixin concentrations on the method validation curve and to evaluate the bixin content present in each sample of the annatto extracts. The images had a rectangular section of the same $R_f$ selected, and the areas in the pixels of the compounds were calculated using the tools <*Rectangular* - Analyze - Gels - Select First Lane - Analyze - Gels - Plot lanes (Plots) - Wand (Tracing) tool (Results, Area)>.

The 3D surfaces of the bands of the different compounds were plotted using the tools <*Rectangular* - Analyze - Gels - Select first lane - Plugins - 3D - Interactive 3D Surface Plot - Select: Filled, (Original Colors, Spectrum LUT or Thermal LUT) and adjusted Grid Size, Smoothing, Perspective, Lighting, Invert, Scale, Z-Scale, Max and Min>.

## 3 Results and discussion

The equipment represented an initial cost of US$ 15,938.24 with the separators and US$ 11,979.84 with the extractor only, using the secondary extraction line (Table 2). The extraction and separation cells, valves and thermostatic baths constituted 60% of the total setup cost of the equipment.

The comparison between the costs of acquisition and setup of the laboratory equipment for extraction with supercritical fluid is the principal result of this work, demonstrating that the design of the SFE unit resulted in low-cost equipment.

Commercial unit I had a higher acquisition cost (Table 2) than the other equipment, although some characteristics of the equipment make a direct comparison impossible. The extractor of commercial equipment I has a volume 5 times larger than that of the other units and an electric pumping system that eliminates the need for an air compressor; additionally, the unit is automated and can use a cosolvent in the extraction process.

Non-automated units have lower acquisition costs relative to automated equipment. For example, Commercial unit II may be automated for an additional US$ 10,850.00 (Table 2).

Comparing the commercial unit of lowest cost, Commercial unit III, to the SFE-0.1L unit without the separators (for both to have the same functionality), the cost of the homemade equipment was 36% of the cost of the commercial unit. Another advantage of the assembly process compared to purchasing commercial equipment is that each project may be adjusted in accordance with the peculiarities of the research objective that will be addressed when using the equipment.

### *3.1 Equipment setup*

Figures 6 and 7 show the SFE-0.1L unit and the component positioning in the aluminum structure. Casters were placed on the lower part of the aluminum structure to facilitate moving the equipment for potential maintenance.

The assembly of the two component support plates could have been achieved with 5-mm-thick aluminum plates to facilitate plate perforation to place their fixing screws. The aluminum plates are light, cost less and easy to handle; moreover, they can support the weight of the two baths in the lower part of the unit and the valve box in the upper part of the unit. Though other materials may be used to support the structural components, stainless steel plates were used in this project because of the availability of this material in our laboratory.

In the lower part of the separators, it is possible to observe an adjustable support for extract collection that occurs sequentially in the V7 and V8 shut-off valves. In this way, it is possible to adjust the height of the support in accordance with the type of bottle that will be used in the extract collection process and to release the extract output by opening only valves V7 and V8.

The extraction and separation cells were aligned with the most external part of the structure and were positioned with the indentation at the front of the structure. This helped reduce the final size of the equipment and the length of the pipes. The equipment measures 57 × 77 × 115 cm.

Another important aspect of the SFE-0.1L unit is that all of the system valves, including the additional valves, such as a backpressure valve for each separator, make it possible to control the cascade of pressures in the separators; the micrometering valve allows control of the solvent flow rate. The use of a backpressure valve for each pressure cell allows precise control of the working pressures. The system that heats the valves using a fluid-flow heat exchanger proved more efficient than the use of electric resistances. The unit has a safety valve that opens at pressures higher than 450 bar, positioned as shown in Figure 5, immediately after the pump and with the relief directed to the rear of the equipment.

### *3.2 Equipment validation*

*Extractor validation using annatto seeds*

The extraction yield of the annatto in the SFE-0.1L unit was 3.8%, comparable to the work of Albuquerque & Meireles (2012), who determined a yield of 3.7%. After 15 minutes of extraction, 74% of all annatto extract was collected; nonetheless, the extraction continued for 80 minutes to complete depletion of the raw material (Figure 8-I). The elevated flow rate (12 g/min) used to validate the equipment resulted in an S/F of 37 at the end of extraction.

Figure 8-II shows the experimental values for extraction performed in the SFE-2×1L (Moraes et al., 2013) and in the SFE-0.1L, plotted as a function of S/F. Though the extractors were 10 times farther apart, the results validated the SFE-0.1 unit because of the overlap of the OECs.

The annatto OEC (Figure 8-I and 8-II) can be fitted to a spline with 3 straight lines ((Meireles, 2008), as shown in Figure 8; the adjusted parameters are given in Table 3. With these results, the SFE-0.1L unit can be considered validated. The SFE-0.1L unit can be expanded to a unit with an extractor of up to 1 liter by adjusting only the supports and the thermal shielding.

**Table 2**. Comparison of the amounts spent assembling the SFE-0.1L unit relative to the cost of acquiring commercial units.

| Equipment | Commercial I | Commercial II | Commercial III | SFE-0.1L* | SFE-0.1L** |
|---|---|---|---|---|---|
| Extractor Volume | 500 mL | 100 mL | 100 mL | 100 mL | 100 mL |
| Number of Separators | 2 | 2 | Not applicable | Not applicable | 2 |
| Rate of flow | 200 g/min | 100 g/min | 0.01-27 g/min | 1.8-30 g/min | 1.8-30 g/min |
| Applicable extractors | 500-1000 mL | 10-500 mL | 5-100 mL | 10-1000 mL | 10-1000 mL |
| Cosolvent | Inclusion | Applicable | Applicable | Applicable | Applicable |
| Operation | Automated | Applicable | Manual | Manual | Manual |
| Price (USD) | $121,991.50 | $48,717.00 | $33,145.36 | $11,979.84 | $15,938.24 |

*SFE-0.1L unit assembled without separators. **SFE-0.1L unit assembled with two separators.

**Table 3**. Parameters from Annatto Extraction Curve.

| | $b_0$ | $b_1$ | $b_2$ | $b_3$ | $c_1$ | $c_2$ | SS |
|---|---|---|---|---|---|---|---|
| Values | 0.015 | 0.4868 | 0.0597 | 0.014 | 4.4435 | 18.046 | 0.0203 |

$b_0$ – Linear coefficient from straight line 1 (CER); $b_1$ – Angular coefficient from straight line 1 (CER); $b_2$ – Angular coefficient from straight line 2 (FER); $b_3$ – Angular coefficient from straight line 3 (DC); $C_1$ – Intercept of the first and second straight lines ($t_{CER}$); $C_2$ – Intercept of the second and third straight lines ($t_{FER}$); SS – Sum of Squares.

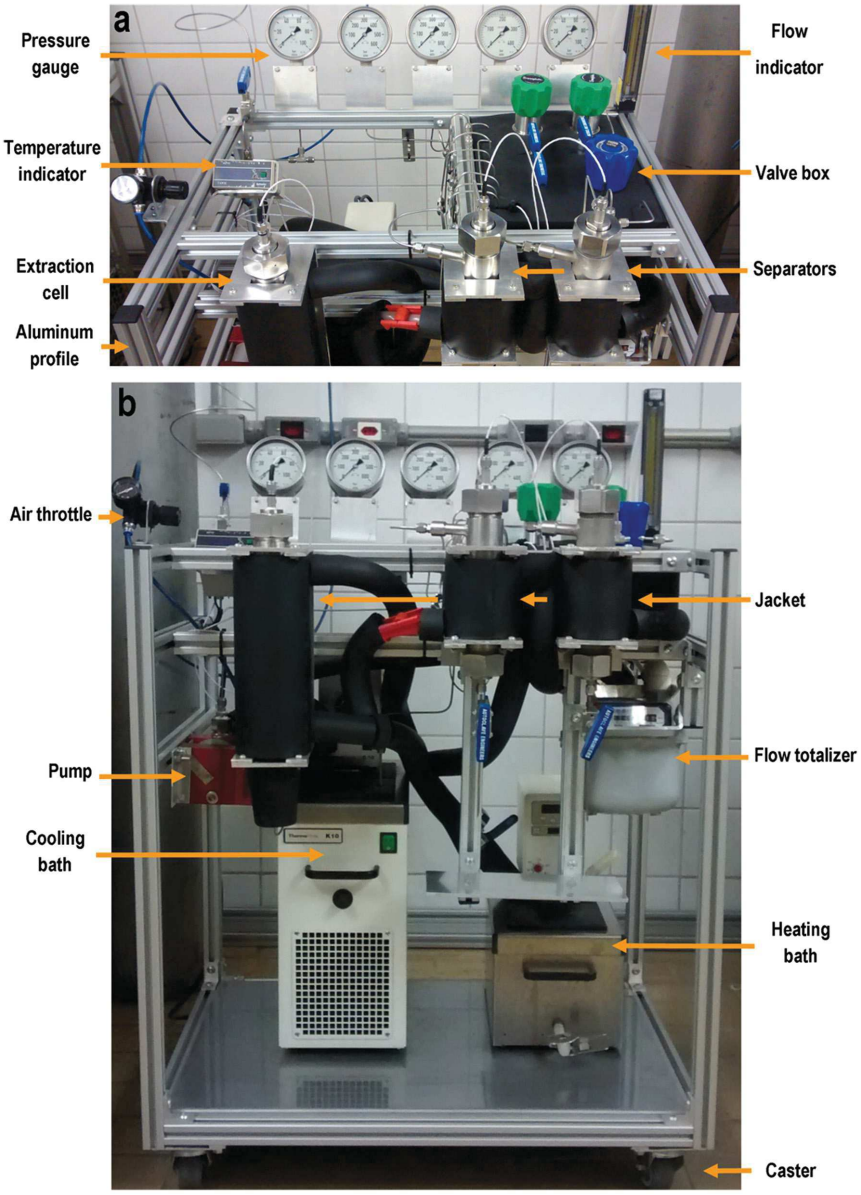
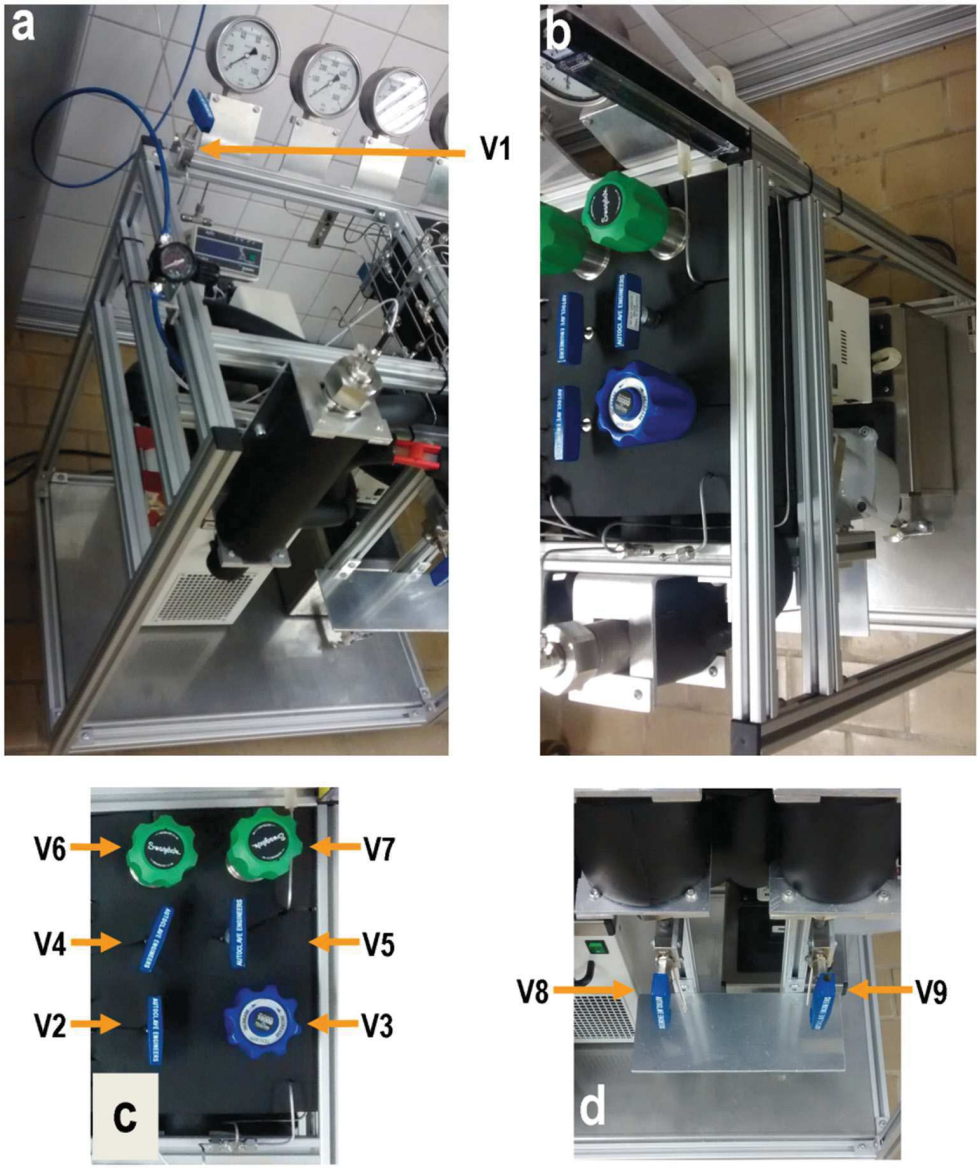



**Figure 6**. Photos of the SFE-0.1L unit, a – top view and b – front view. validating the equipment.

*Validation of the separators using fennel*

The commercial equipment from Thar Technologies (model SFE-2×5LF-2-FMC, Pittsburgh, PA, USA) has 2 extractors of 5 liters and 3 separators with volumes of approximately 500 mL. Considering the possibility of using a larger capacity extractor in the SFE-0.1L unit, the separators were designed with volumes of 90% of that of the extractors. The reasoning behind this choice was to ensure that the separators would hold as much extract, in terms of capacity, as would be obtained from a raw material with up to 30% of solute. Considering that, on average, each 100 mL extraction cell may contain approximately 70 grams of raw material for a 3.8% yield, approximately 2.7 grams of extract would be obtained. Otherwise, for a raw material having 30% of soluble material using a 1-liter extractor capable of processing 700 g of raw material, the extract mass would be ~ 270 grams. Therefore, in this case, the separators must be capable of holding this amount of extract, and separators of approximately 100 mL would be required. This estimate takes into account the fact that the extract is continuously collected. Therefore, for the SFE0.1L unit, 90 mL separators were used, representing 90% of the capacity of the extractor; the larger capacity of the separators thus leaves open the possibility of using a larger extractor.




| a | | c | |
|---|---|---|---|
| V1 | $CO_2$ Blocking Valve | V2,V4 | Extractor Inlet and Outlet Blocking Valves |
| d | | V5 | Micrometering Valve |
| V8 | Separator 1 Outlet Blocking | V3 | Back Pressure Regulator for Extraction |
| V9 | Separator 2 Outlet Blocking | V6,V7 | Back Pressure Regulator for Separation |

**Figure 7**. Photos of the SFE-0.1L valves, (**a**) left side view; (**b**) right side view; (**c**) top view and (**d**) front view.

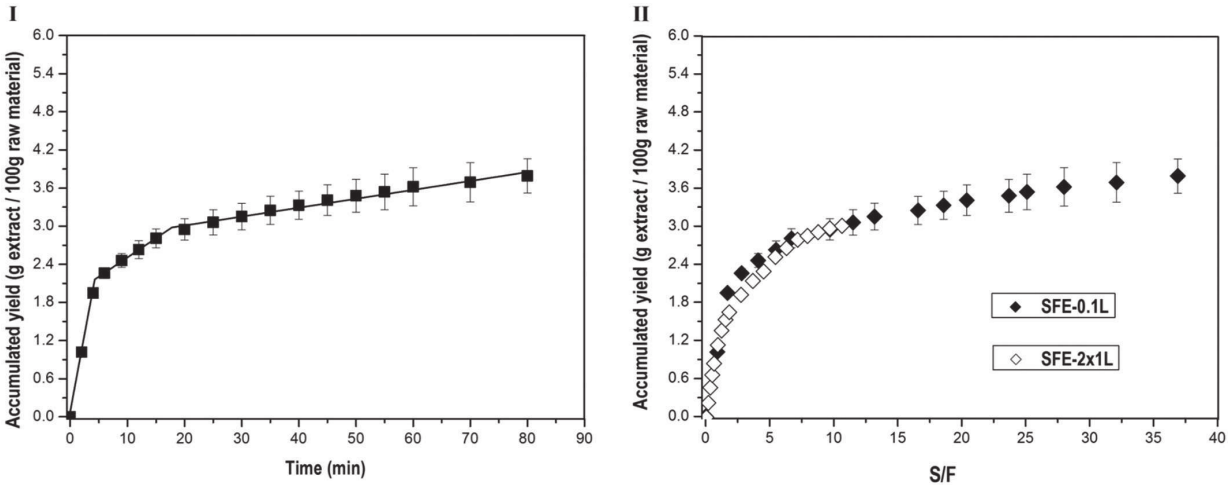



**Figure 8**. Overall extraction curve obtained on SFE-0.1L equipment and kinetic curves of validating the equipment.

Another important aspect to be considered in the setup of the equipment is that many of the raw materials have a group of majoritarian components. In other words, the division of the extract in the separators may only be 3% for one of the groups of compounds; therefore, the other 97% should be retained in the other separator. The work of Coşge et al. (2008) evaluated the composition of the sweet fennel seed oil and found that sweet fennel has a relationship of 93:3 of oleoresin:essential oil.

There are little data in the literature on the separator volume compared to the extractor's capacity, and the actual construction of equipment at laboratory scale can now serve as an initial parameter for pilot-scale applications.

The fennel extraction in the SFE0.1L unit resulted in a yield of 2.8% (g extract/100 g of ground seeds). As discussed in the next section, this extract mass contained 97.5% of a lipidic fraction collected in the first separator; the remaining 2.5% was the volatile oil collected in the second separator. Stefanini et al. (2006) determined yields of 1.54 to 2.2% using hydrodistillation to obtain fennel volatile oil; both the yield and composition varied with the crop conditions. The higher yield obtained in this work is because $CO_2$ extracts the lipidic fraction in addition to the volatile oil.

### *3.3 Analyses of extracts*

The method used to determine the bixin concentration showed linearity for concentrations between 0.6 to 9.6 μg, as shown in Figure 9E, and had a repeatability that varied by 3.4%, as observed by comparing the areas of the pixels of Figures 9A and 9B.

In Figure 10, the presence of bixin is observed in all of the extracts collected during the extraction; nonetheless, it is important to acknowledge that annatto extract contains other carotenoids that are present in the seeds (Mercadante & Pfander, 2001). Although TLC is a technique that is used mainly in qualitative analysis, it is possible to observe a tendency for the bixin concentration to increase throughout extract collection at an $R_f$ of 0.1, as shown in Figure 10A and plotted in Figure 11. In a similar manner, Albuquerque et al. (2015) found an increase in bixin concentration as a function of extraction time.

Validating the bixin quantification method by TLC using the ImageJ software made it possible to estimate the carotenoid concentration as a function of the extraction time. In this way, in addition to polling by separation of the present compounds, it was possible to estimate the bixin extraction curve.

Albuquerque & Meireles (2012) obtained annatto extract using a commercial SFE unit Spe-ed (Laboratory System 7071, Applied Separation, Allentown, USA) with a 0.29 L extractor. The data obtained from the extraction on the SFE-0.1L unit with the same raw material is an additional important aspect in validating the equipment: not only was a similar OEC determined, but the extract composition during the extraction was also similar to that obtained in the commercial unit (Figure 8).

In the images of Figure 10, the $R_f$ of 0.98 for all of the annatto extracts showed some distinct pixel intensities on the plate at 366 nm and 245 nm and, with the use of the reagent AS, revealed the presence of these compounds in all of the samples in the visible range. The type of fluorescence of image C is not characteristic of either terpenoids or polyphenols (Wagner & Bladt, 2001). The plate without developer presented an increased $R_f$ concentration with increasing extraction time, which may indicate that there are different compounds eluted in this same $R_f$, characteristic because they have less affinity with the mobile phase hexane:ethyl acetate:formic acid (79:18:3).

For the $R_f$ 0.89 and 0.55 (Figure 10A), the concentration of the compounds increased with extraction time; the same behavior was observed at $R_f$ of 0.10 and 0.35 for the plate in Figure 10B.

The use of the AS developer made it possible to visualize the various compounds that could not be observed in Figure 10A, which appear in Figure 10B at $R_f$ values of 0.18, 0.28, 0.47, 0.63 and 0.79. The AS reagent is a developer of volatile oils that doubles

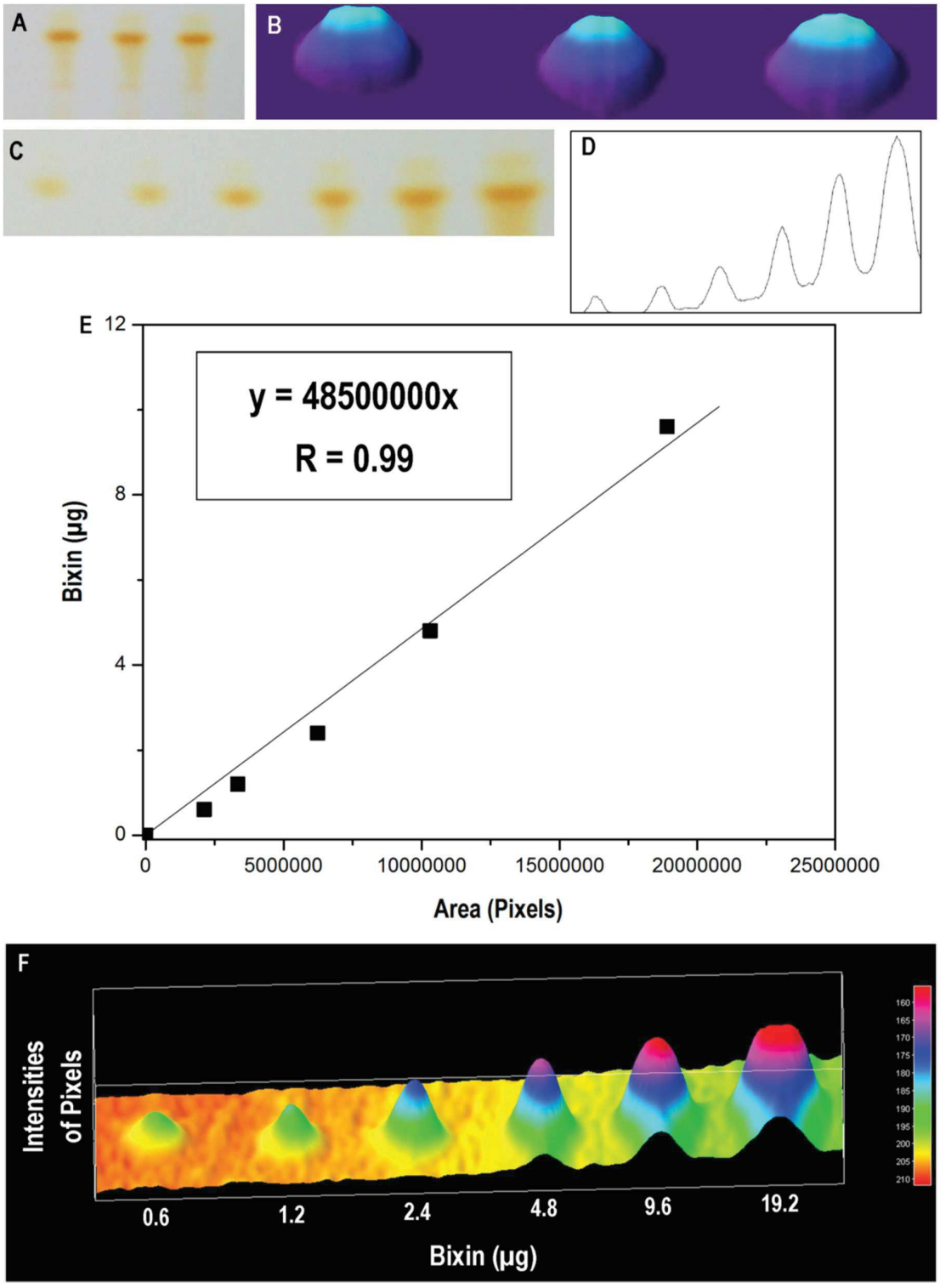



**Figure 9**. Validation steps of the bixin determination method. (**A**) Image of plate chromatography refers to method repeatability; (**B**) Image **A** manipulated with ImageJ (Interactive 3D Surface Plot - Thermal LUT); (**C**) Image of the chromatographic plate refers to method linearity; (**D**) Image **C** manipulated with ImageJ (Plot Lanes); (**E**) Graphic of bixin concentrations by areas of the pixels obtained by ImageJ; (**F**) Image **C** manipulated with ImageJ (Interactive 3D Surface Plot - Spectrum LUT).

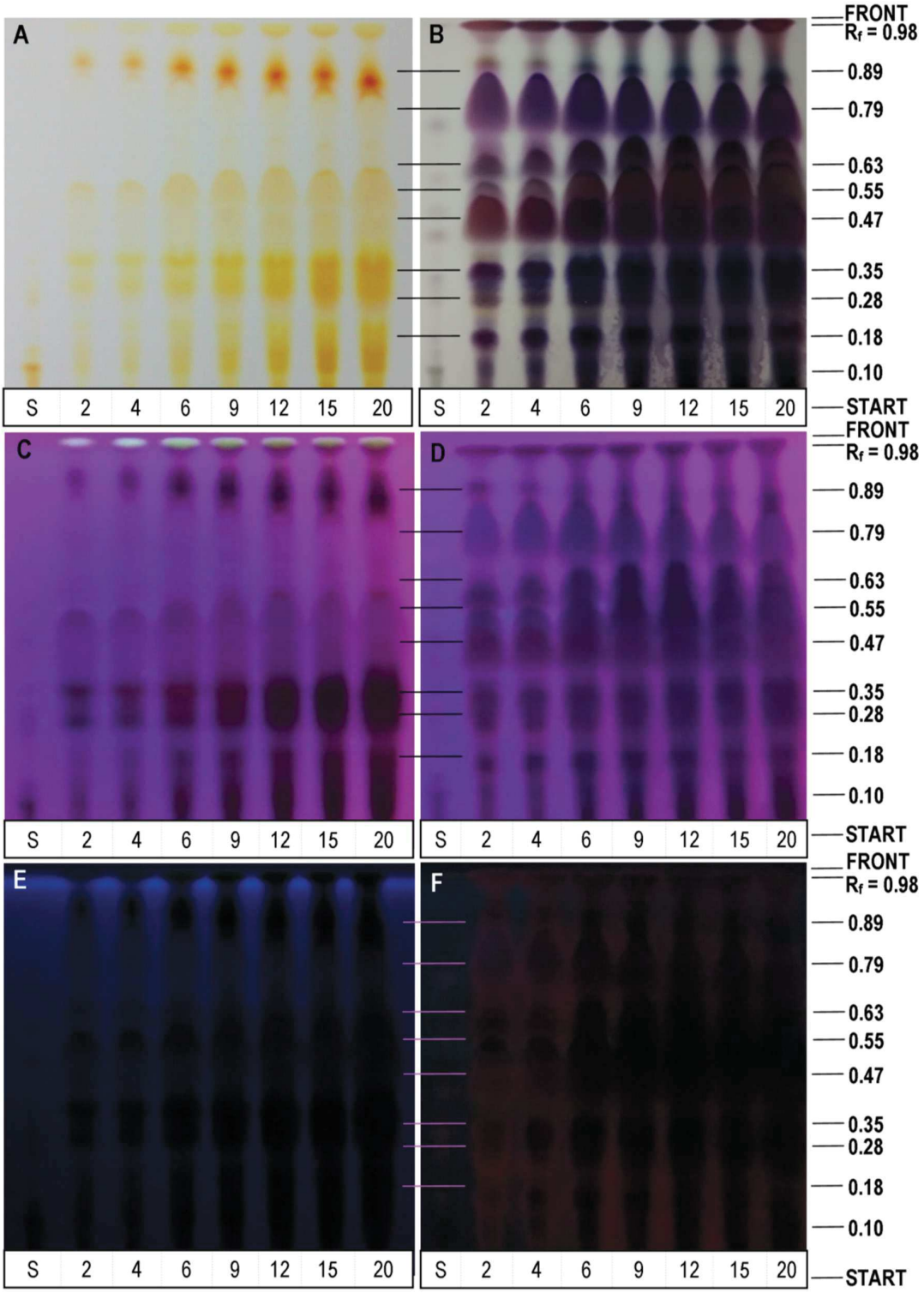



**Figure 10**. Image of the chromatographic plates obtained from the elutions of the extracts from the kinetic points of Figure 8 (Lower codes: S - Bixin Standard and the respective numbers in minutes on the kinetic points). Photo **A** (Without Developer - Visible), photo **B** (With Developer - Visible), photo **C** (Without Developer - 366 nm), photo **D** (With Developer - 366 nm), photo **E** (Without Developer - 254 nm) and photo **F** (With Developer - 254 nm).

the quantity of compounds in the visible range for the annatto extracts, with the blue, green, red and brown colorations being characteristic of the essential oils (Wagner & Bladt, 2001); this confirms the presence of geranilgeraniol in annatto extracts.

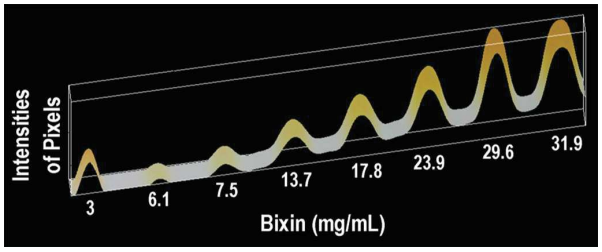



**Figure 11**. Image selected from Figure 10A ($R_f$ = 0.1) manipulated with ImageJ (Interactive 3D Surface Plot - Original Colors).

The fennel extracts collected (Figure 12) in Separator 1 (I) and Separator 2 (II) have different compositions. The chromatography plate shows the different compositions of the extracts retained in each separator. The blade (*) plotted on the 3D surface reinforces the differences in extract compositions, where (*) $R_f$ = 0.43 corresponds to the fenchone present in the extract obtained in Separator II.

The method of Wagner & Bladt (2001) for developing volatile oils using concentrated sulfuric acid found that the fenchone to be sprayed should be revealed by a yellowish coloration at $R_f$ ~ 0.5, if it is present at concentrations higher than 100 µg. After the chromatographic plate was sprayed with this reagent, the plate presented a coloration close to that of fenchone, which is difficult to visualize at low concentrations. However, by using the Invert tool in ImageJ, which inverts image colors, it is possible to more clearly identify the compound.

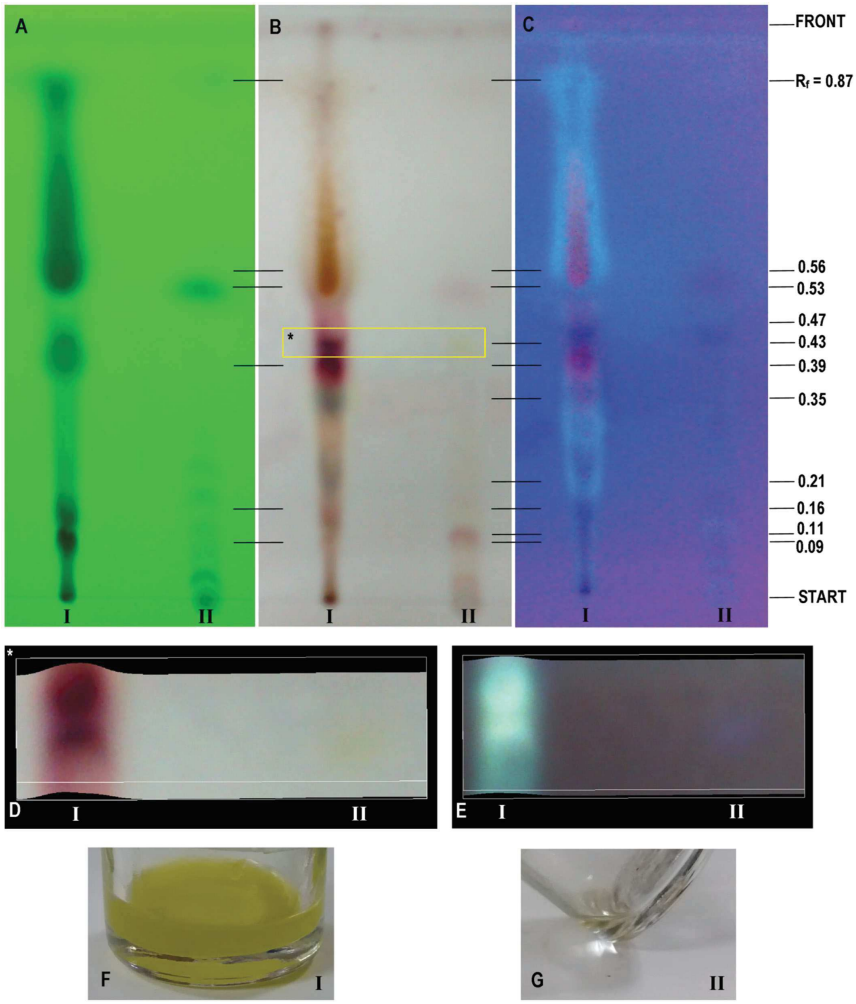



**Figure 12**. Images of the chromatogrpahic plates and of the extracts obtained from the fennel seeds from separators I and II. Photo **A** (Without developer - 254 nm), photo **B** (With developer - Visible), photo **C** (With Developer - 366 nm), photo **D** (Image **B*** manipulated ImageJ - Interactive 3D Surface Plot - Original Colors), photo **E** (Image **D** manipulated with ImageJ - Invert), photo **F** (Extracted from the first separator) and photo **G** (Extract from the second separator).

Of the 2.8% yield of fennel extract, 97.5% was a waxy extract collected in the first separator, and 2.5% was an oily extract collected in the second separator. As observed in Figure 12 in the images of extracts I and II, these extracts presented distinct visual characteristics, with these being the first indicators showing the effectiveness of the separators. The work of Coşge et al. (2008) analyzed the oil of sweet fennel and found 3% essential oil, with anethole, estragole and fenchone being the predominant components of this fraction, and found 93% of monounsaturated fatty acids in the oleoresin fraction.

The extract collected in the first separator is formed by an oleoresin that also contains red-violet anisaldehyde, $R_f = 0.39$, and anethole, $R_f = 0.87$. The extract from the second separator is formed exclusively by essential oils and presented fenchone, $R_f = 0.43$ (Figure 12E), and two other compounds, $R_f = 0.53$ (Figure 12A) and $R_f = 0.11$ (Figure 12B).

## 4 Conclusions

The assembled equipment may be used as a standard for a low-cost SFE unit for the formulation of projects to construct new extraction units. The extractor is adequate for use in obtaining extracts of diverse raw materials, and its separators can function to obtain fractionated extracts. The validated method for bixin quantification and the procedure adapted for the production of a bixin pattern represent a low-cost option for annatto extract analysis. The description of the use of the free ImageJ software may be adapted for analysis of other images that need precise measurements, whether they are intended to calculate $R_f$ or not, such as the use of the tools to calculate areas from parts of images.

## Acknowledgements

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**Appendix A**. SFE-0.1L Unit Standard Operating Procedures (SOPs). Campinas-SP, July of 2015.

## Standard Operating Procedure

Knowledge of all the components of the unit is an important step in operating the equipment. SFE-0.1L unit consists of the following:

- 1 structure formed by aluminum profile 30 × 30 mm (Sistema, Amparo, Brazil)
- 2 swivel casters without brakes connected to the structure (Colson, 3"s, Araucária, Brazil)
- 2 swivel casters with brakes connected to the structure (Colson, 3"c, Araucária, Brazil)
- 1 aluminum plate (Autic, Campinas, Brazil) that serves as the foundation for the heating and cooling baths (770 × 570 × 2 mm)
- 1 stainless steel plate that serves as the foundation for the valve box with heating system (Maq'nagua, Serra Negra, Brazil)
- 1 valve panel formed by a stainless steel plate with a size of 320 × 270 × 2 mm (Maq'nagua, Serra Negra, Brazil)
- 1 heating valve box (310 × 260 × 120 mm) for the valve panel with fluid inlet and outlet with diameter of 22 mm (Maq'nagua, Serra Negra, Brazil)
- 1 backpressure valve (Tescon, 26-1700, Sorocaba, Brazil)
- 1 backpressure valve (Tescon, 44-2200, Sorocaba, Brazil)
- 1 backpressure valve (Tescon, 44-1800, Sorocaba, Brazil)
- 1 air pressure regulator (Norcren, R07-100-RNKA, São Paulo, Brazil)
- 1 safety valve (Swagelok, SS-4R3AS, São Paulo, Brazil)
- Tube adapter, tube OD 1/8" e NPT female 1/4" (Swagelok, SS-400-6-2, São Paulo, Brazil)
- 1 air-driven $CO_2$ pump (Maximator, M-111L, Nordhausen, Germany)
- Tube 1/8" OD of 0.89 mm length (Fopil, A.I.ASTMA269TP316S, Campinas, Brazil)
- Tube adapter - Union Tee Connector, tube OD 1/8" with ferrule (Fopil, ASTMA276TP316, Campinas, Brazil)
- Tube adapter - Straight Union, tube OD 1/8" with ferrule ASTM A276TP316 (Fopil, A.I.ASTMA269TP316S, Campinas, Brazil)
- Tube adapter - tube OD 1/8" e NPT Male 1/4" ASTM A276TP316 (Fopil, A.I.ASTMA269TP316S, Campinas, Brazil)
- Tube adapter - union tee male branch NPT 1/4" (Fopil, ASTMA276TP316, Campinas, Brazil)
- Cylinder outlet connection (Hoke 7115F4Y, Spartamburg, EUA)
- Blocking valve (Autoclave Engineers, 10V2071, PA, USA)
- Micrometering valve (Autoclave Engineers, 10VRMM2812, PA, USA)
- Particulate filter (Swagelok, série F, São Paulo, Brazil)
- 2 pressure gauge - 100 bar (WIKA, EN8371-1, Klingenberg, Germany)
- 2 pressure gauge - 600 bar (WIKA, EN8371-1, Klingenberg, Germany)
- 1 pressure gauge - 400 bar (WIKA, EN8371-1, Klingenberg, Germany)
- Insulation sheets - 13 mm (Epex, Vidoflex M2, Blumenau, Brazil)
- 1 temperature indicator 173.2 K — 473.0 K (Pyrotecautomação, T4WM-TP100, Campinas, Brazil)
- 3 thermocouples - 50 mm × 1.2 m (Pyrotecautomação, PT 100, Campinas, Brazil)
- 2 thermocouples - 150 mm × 1.2 m (Pyrotecautomação, PT 100, Campinas, Brazil)
- 1 separator with heating jacket, 90 mL
- 1 separator with cooling jacket, 90 mL
- 1 extractor with heating jacket, 100 mL
- 1 ramp clamp (Keck SK, Max. tubing o.d. 1/4 in. / 6 mm, Munich, Germany)

- 2 ball valve (Japi, globo, Jundiaí, Brazil)
- Flowmeter (Cole Parmer, PMR1, IL, USA)
- Flow totalizer (Itrón, G25, Americana, Brazil)
- Silicone hose (Sinergia, 200 × 200, Campinas, Brazil)
- Insulation tube 1/8" (Armaflex, AFM-10, Campinas, Brazil)
- Insulation tube Ø - 22 mm (Isolan, Campinas, Brazil)
- 2 serpentine tubes 1/8"
- 1 hose serpentine
- 1 steel plate (420 × 350 × 2 mm)
- 2 hose npt adapter (female 22 mm) 1/2" (Amanco, Campinas, Brazil)
- 2 Hose Tee connector 1/2" (Amanco, Campinas, Brazil)
- 1 extractor support structure (Autic, Campinas, Brazil)
- 4 separators support structures (Autic, Campinas, Brazil)
- 1 aluminum plate 110 x 270 x 5 mm (Autic, Campinas, Brazil)
- 1 heating bath (1500 W) 243 K — 373 K (Thermo Haake, C10, Eindhoven, Holland)
- 1 cooling bath (2000 W) 278 K — 368 K (Thermo Haake, DC30/DL30, Eindhoven, Holland)

Each topic presented in the SOP is numbered according to the sequence of the unit operational steps and may be followed by the underlined word "Caution", with an explanation of what should be checked.

## Standard procedure to turn on and operate the SFE-0.1L unit

1) Turn on the air conditioning of the extraction room for approximately 10 minutes before starting work on the unit to operate at constant room temperature; record this value;
2) Record the value of the flow totalizer (Figure 1A);

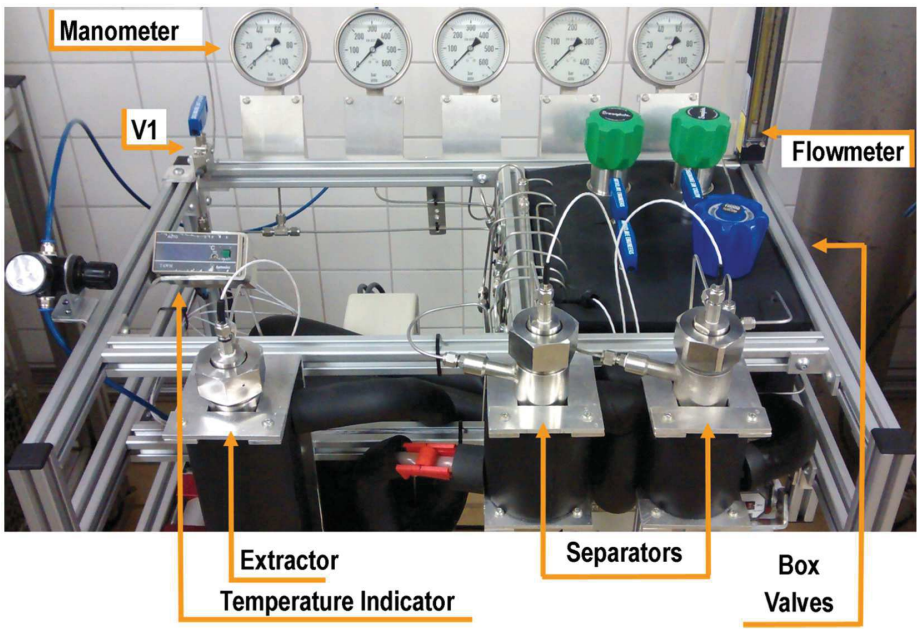



**Figure 1A**. Unit SFE-0.1L, front view.

3) Connect the power cables of the temperature indicator, thermostatic bath and heating bath in voltage and amperage indicated in the plugs (Figures 1A and 2A);

4) Record the temperature of the temperature indicator (Figure 2A);

5) Release the pressure of the compressed air to the regulator (Figure 1A);

6) Close the blocking valve V1 (Figure 2A);

7) Open the $CO_2$ cylinder valve and the ball valve of the cylinder connector that carries the $CO_2$ to the tubes (Figure 3A);

8) Close the blocking valve V3 (Figure 4A);

9) Turn on the thermostatic bath until it reaches operating temperature. The maximum working temperature must be 268 K and the minimum 255 K. The thermostatic bath takes approximately 45-60 minutes to reach the temperature;

10) Disconnect the 1/8" tube of the hose t connector connected to the upper part of the extractor with a 55 mm wrench with a movement of 90° counterclockwise; after this rotation no longer use the wrench, but finish with the hands (Figure 5A). Caution: If is not possible to finish opening using the hands, the thread has been damaged. Send the cover and the thread for repair before it is no longer possible to open it using a 55 mm wrench. To open the unit cell, it must be under internal pressure conditions similar to the environment to prevent damage to their threads. The covers are made of the same material as the threads, and they could be caught in the thread if torque were applied with a key while the cell was pressurized. At the slightest sign of trouble while opening, send the parts for repair;

11) Place the desired raw material inside the extractor;

12) Place the Teflon gasket into the cell and then screw the cover with your hands until the point where is necessary to use the wrench. Place the center output of the hose's T connector that connects the cover to the tubing and the thermocouple with a 45 ° angle relative to the tube that connects the 1/8 tube (Figure 5A). Exert torque on the cover clockwise with a 55 mm wrench until the connection aligns with the 1/8" tubing and screw the tubing into the hose's T connector;

13) Open the blocking valve V1; the cylinder manometer pressure should reach 60 bar (Figure 6A), in case the cylinder is full. (Only use the cylinder with pressure between 60 and 50 bar);

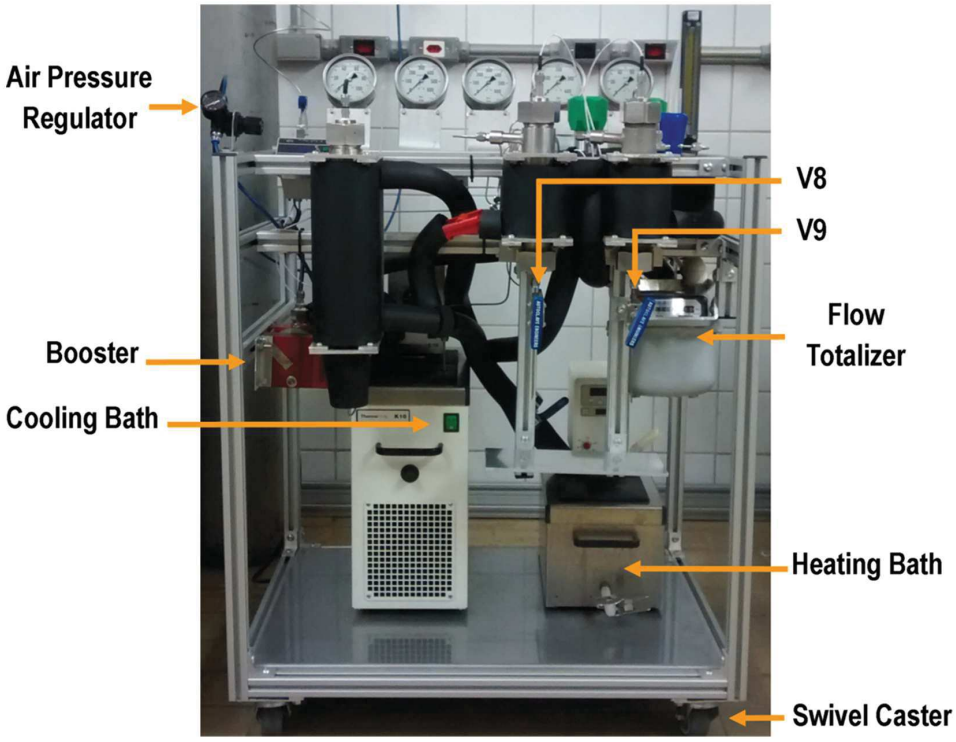



**Figure 2A**. Unit SFE-0.1L, perspective view.

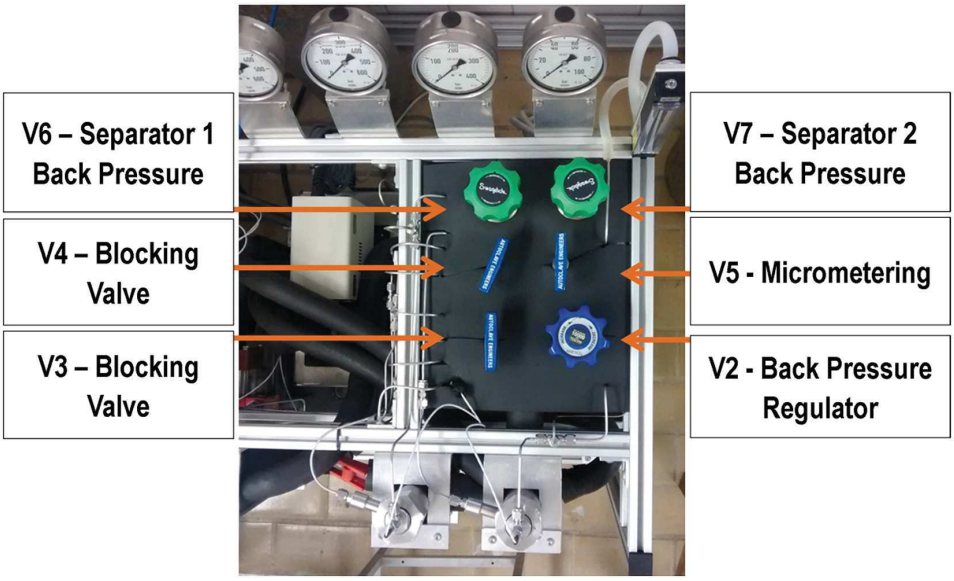



**Figure 3A**. Box valves.

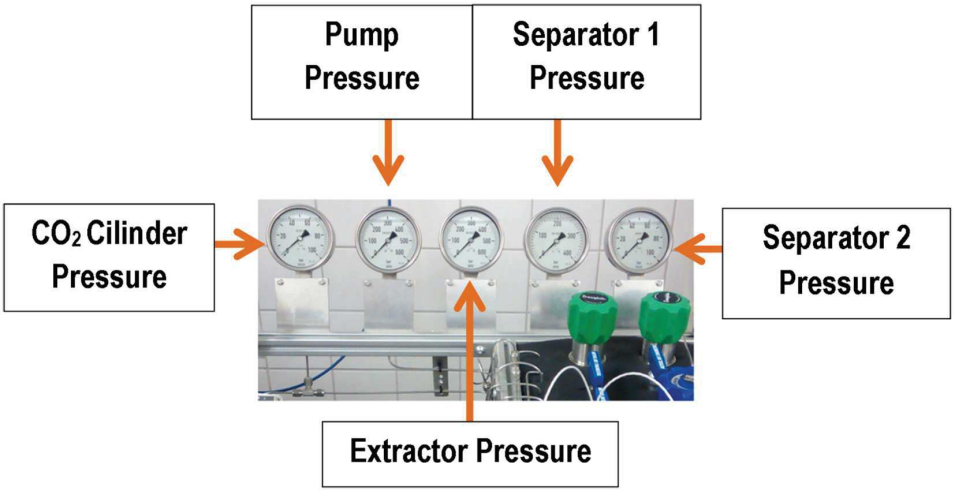



**Figure 4A**. Manometers.

14) Before turning on the heating bath (Figure 1A), the water level should be checked. Additionally, observe if the water is clean (Figure 7A); because the jacket is made of copper, the fluid should be periodically changed to avoid rusting of the thermocouple, which could affect the temperature control. Indeed, some dirty particles could damage the pump of this component. Caution: The fluid level of the heating bath should be higher than the black plastic connected to the tube and always lower than the cover of this component (Figure 7A). If the fluid level is not between the indicated positions, the bath will turn off automatically and the fluid will return to the valve panel, causing an overflow. To avoid this inconvenience, two ball valves at the base of the bath pump output (Figure 8A) and a type of hose valve with an open body were installed;

15) Turn on the heating bath. (This heating bath interchanges thermal energy with the extractor jacket, with the jacket of the first separator and with the valve panel). Because of the specifications of the backpressure valves, the maximum temperature of the heating bath should be 353 K. Check that the bath temperature reaches the desired temperature in the extractor. If necessary during the extraction, regulate the bath temperature to keep the temperature of the thermocouple at the extractor outlet constant;

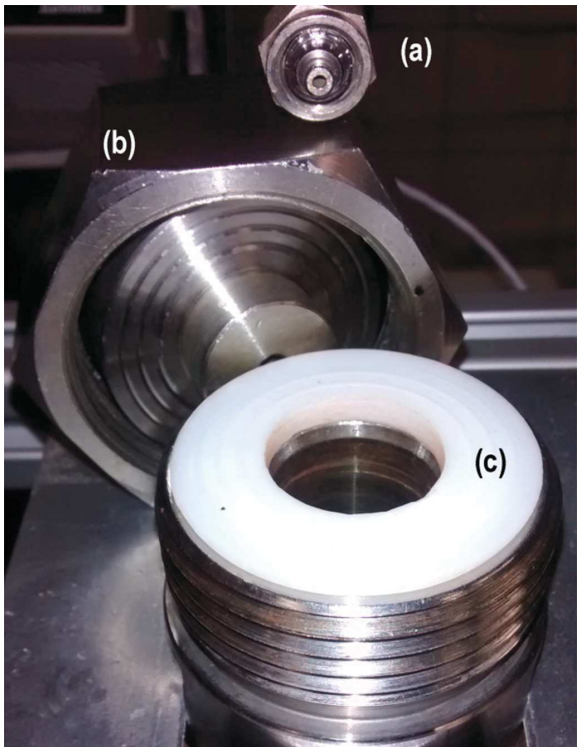



**Figure 5A**. Extractor components. (a) $CO_2$ inlet 1/8 tube; (b) Extractor - 55 mm; and (c) Teflon.

**Figure 6A**. CO2 ball valve.

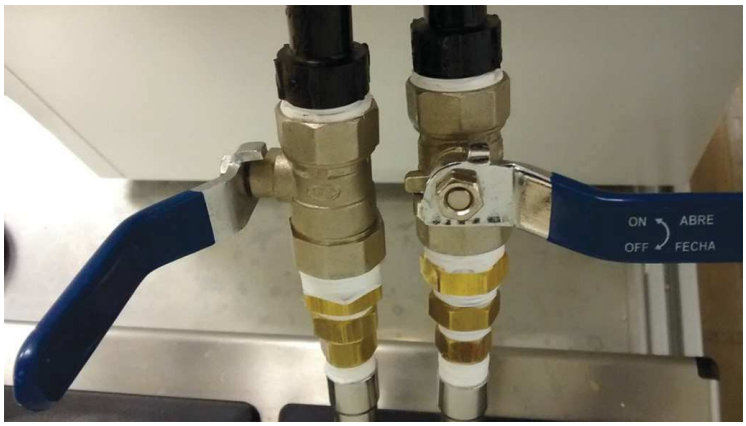



**Figure 7A**. Ball valve for water control.

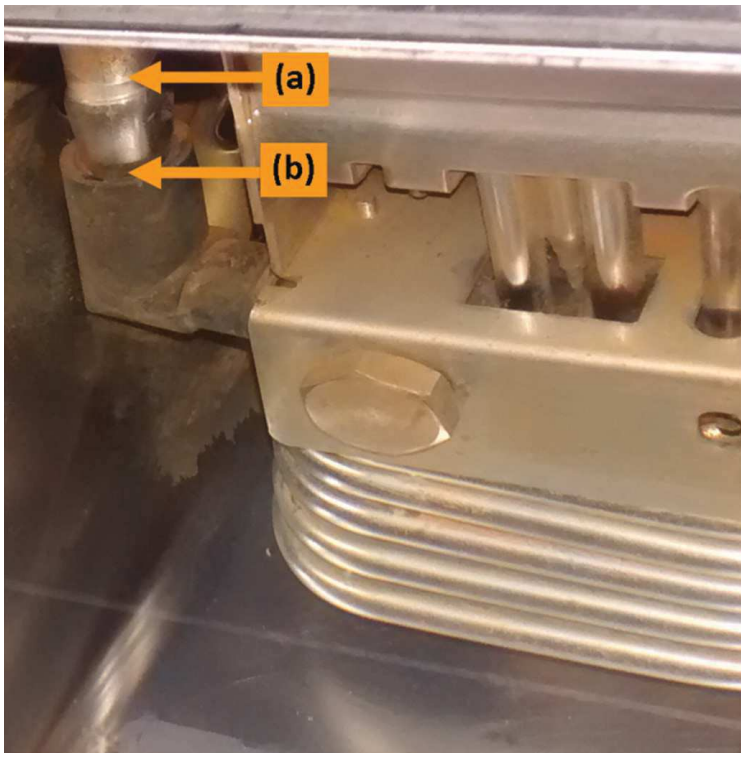



**Figure 8A**. Bath level. (a) Full mark, do not overfill; (b) Add mark, add water when level is at or below this mark.

16) Then, pressurize the tube that connects the air-driven $CO_2$ pump to the valve V3. Slowly release the air pressure to the air-driven $CO_2$ pump, which should begin to move its piston at 2.5 bar of pressure with the pump regulator gauge (Figure 1A). Because the backpressure valve is still fully open, the pressure should not rise in the second manometer and the flow corresponding to the excess pressure should be recirculated to the tubing before the air-driven $CO_2$. At this point, the backpressure valve should start to slowly close; the pressure in the second manometer will rise. The air-driven $CO_2$ pump should begin to pressurize (pump) slowly and then more pressure should be liberated from the compressed air regulator until the desired pressure is reached and the $CO_2$ is recycled by the backpressure valve. (Caution: the air-driven $CO_2$ pump should work constantly to avoid pressure changes in the compressed air that cause changes in the extractor pressure. Thus, after the initial adjustment, the equipment should work constantly until the end of the extraction process without the need for controlling the pressure of the compressed air or the back pressure, Figure 4A);

17) Once the pump has been working constantly for some seconds, recycling the flow corresponding to the excess pressure after the pump, the blocking valve V4 should be closed (Figure 4A). Then, slowly open the blocking valve (Figure 4A) until the pressures in the extraction and pump manometers are the same. Caution: Do not open the blocking valve V3 suddenly; if this happens, the pressure after the air-driven $CO_2$ pump will fall sharply and it will pump unevenly, which may cause undesired operation;

18) Count 20 minutes of static time;

19) The micrometering valve V5 must be closed carefully. Do not close it totally and damage its needle. Caution: The micrometering valve should never be completely closed; for this reason, a blocking valve is installed in the same line for the sole purpose of performing this function. In this way, when the blocking valve is opened, the micrometering valve V5 is already in the working position and only requires an initial adjustment to maintain the flow rate;

20) After the static time and with the micrometering valve already adjusted to control the flow (this point corresponds to approximately 45° of rotation before the flow is blocking; never completely close the valve to avoid damaging the needle), release the blocking valve V3;

21) Record the beginning of the extraction time;

22) At this time, the flow adjustment should be performed only through the micrometering valve to keep it constant during the entire process. Caution: Considering that the fluid should pass across two separators before entering the flowmeter, a few seconds are spent before observing some change in the flowmeter caused by a position change in the micrometering valve. To avoid damage to the needle, it is necessary to wait for the response in the flowmeter before adjusting the valve V5 again (Figure 4A). Then, wait for approximately 15 seconds to evaluate if the flow in the flowmeter is constant;

23) Once the flow rate has been regulated, begin to close the backpressure valve V6; this valve should be closed by turning it clockwise 45° until the desired pressure in the manometer is reached (Figure 6A). Caution: This sort of valve has better precision when it is closing than when it is opening; thus, do not pressurize the system beyond the working pressure;

24) Then repeat the procedure described in 23) with the backpressure valve V7, which should be regulated with less pressure than the first separator. Never use excessive torque on any valve, especially on the backpressure valves; their springs are very sensitive and approximately 1200 ° rotation is enough to reach pressures of 200 bar;

25) Following the specified procedures, the unit should be operated without the necessity for any adjustment. If there is some variation in the flowmeter, repeat 22) to 24) while the extracts are being stored in the separators.

**SFE-0.1L unit shutdown procedure:**

1) Once the extraction time is finished, close the blocking valve V4 (Figure 4A);
2) Close the compressed air feeding valve (Figure 1A);
3) Close the blocking valve V3 (Figure 4A);
4) Close the $CO_2$ cylinder valve (Figure 4A);
5) Turn off the thermostatic bath (Figure 1A);
6) Record the flow totalizer value (Figure 1A);
7) The pressure should fall in the extractor when the air-driven CO2 pump has stopped. Then carefully open the valves V4 and V5, trying to maintain a constant flow rate, to depressurize the system. Once the extractor pressure is the same as that in the first separator, open the micrometering valve V5 totally;
8) Gradually open the backpressure valve V6, maintaining the flowmeter at the same process flow rate until the pressures equalize in both separators;
9) Gradually open the back pressure valve V7, maintaining the flowmeter at the same process flow rate until 5 bar is reached in the three cells, extractor, separator 1 and 2;
10) Recover the extracts using proper flasks, previously weighed;
11) Adjust the collector base extract based on the flask height;
12) Open the valves V8 and V9; the remaining pressure will facilitate the recovery of the extract (Figure 1A).
13) Record the flow totalizer value corresponding to the end of the depressurization process;

14) Open the 1/8" tubing that connects with the hose T connector. The pressure will fall to the ambient pressure. With the extractor still warm, open the top cover and remove the raw material;

15) Turn off the heating bath and immediately close both blocking valves at the pump outlet (Figure 8A) to avoid heating fluid reflux;

16) Perform the cleaning procedure described in the SFE-0.1L Unit Standard Cleaning Procedure;

17) Disconnect the power cables of the temperature indicator, thermostatic bath and heating bath.

**Appendix B**. SFE-0.1L Standard Operating Procedures (SOPs) of the Secondary Extraction Line. Campinas-SP, July of 2015

## Standard Operating Procedures (SOPs) for the secondary extraction line

To perform studies in the SFE-0.1L unit without using the separators or if the objective is to build the unit without installing the separators, a secondary extraction line was developed. This line consists of a 1/8" tube connected to the outlet of the micrometering valve that should be inserted into a collecting flask connected after the flowmeter and flow totalizer.

Figure 1B shows the tube used in the procedure, with an angle to allow the tube to exit the valve panel; the extract is thus recovered. The details of the secondary extraction line are shown in Figure 2B; all equipment components are arranged in a flow diagram.

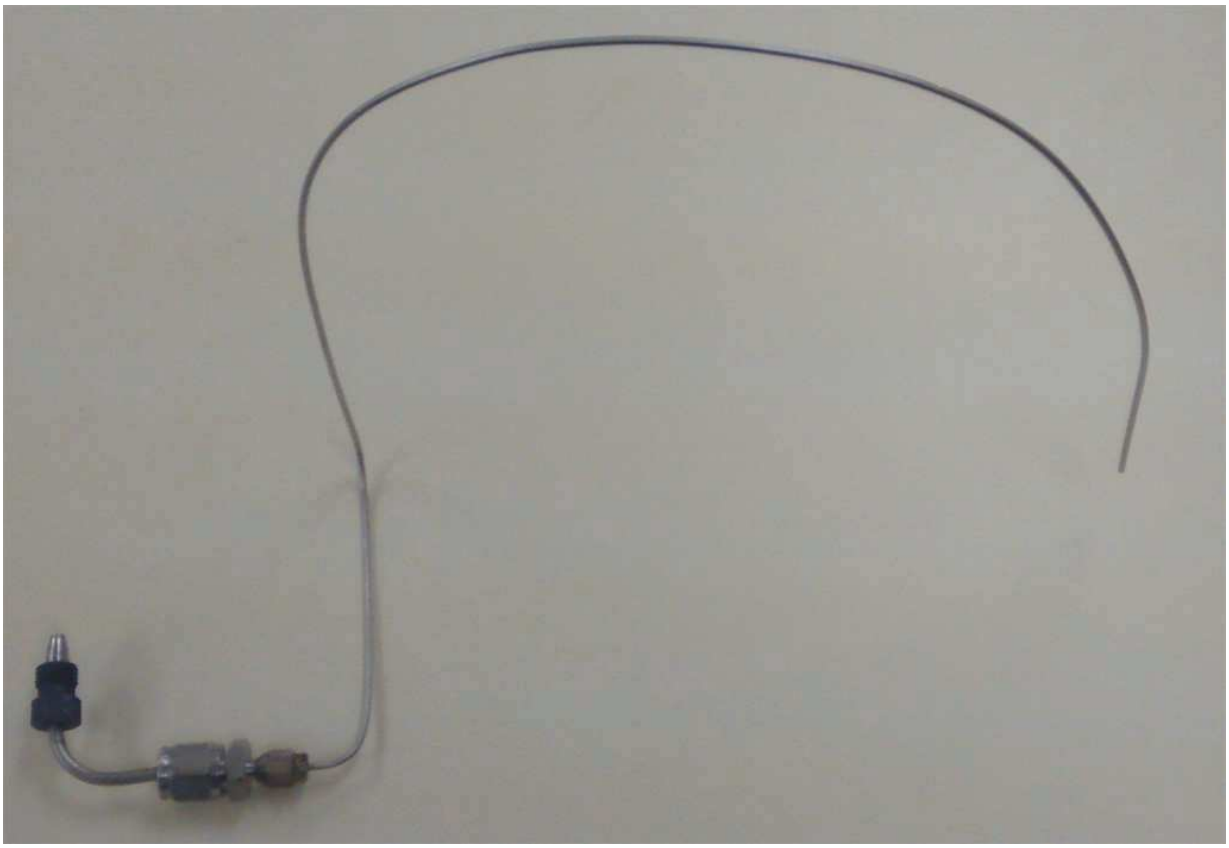
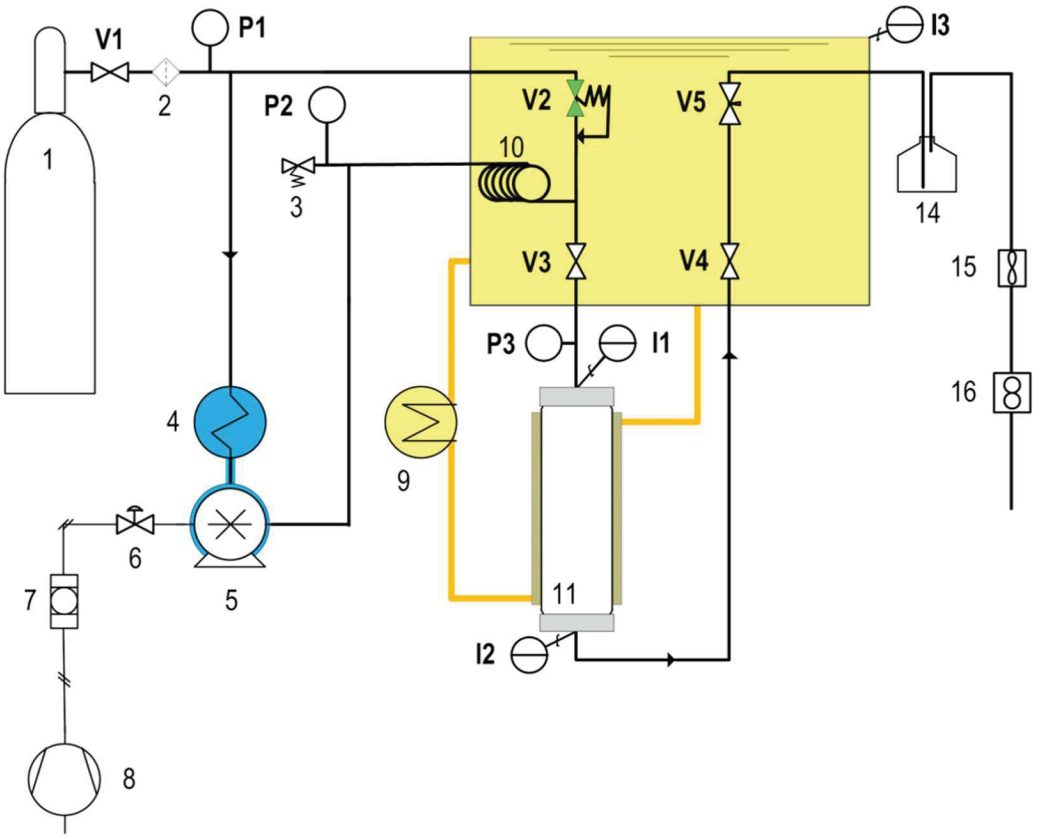


**Figure 1B**. Line adapter for secondary extraction.




| List of equipments | | | | List of valves | |
|---|---|---|---|---|---|
| 1 | $CO_2$ reservoir | 11 | Extractor | V(1,3,4) | Blocking valve |
| 4 | Cooling bath | 14 | Extrac collecting vessel | V5 | Micrometering valve |
| 5 | Air-driven $CO_2$ pump | List of instruments | | V2 | Back pressure (600 bar) |
| 7 | Air filter | 15 | Flowmeter | 3 | Safety valve |
| 8 | Air compressor | 16 | Flow totalizer | 6 | Control (air flow) |
| 9 | Heating bath | I(1,2,3) - Temperature Indicator | | | |
| 10 | Serpentine tube | P(1,2,3) - Pressure gauge | | | |

**Figure 2B**. Diagram of the secondary extraction line.

## Standard Operating Procedure

The knowledge of all unit components is a key factor in proper operation of the equipment. The secondary extraction line consists of the following:

- 1 structure formed by an aluminum profile 30 × 30 mm (Sistema, Amparo, Brazil)
- 2 swivel casters without brakes connected to the structure (Colson, 3"s, Araucária, Brazil)
- 2 swivel casters with brakes connected to the structure (Colson, 3"c, Araucária, Brazil)
- 1 aluminum plate (Autic, Campinas, Brazil) that serves as the foundation for the heating and cooling baths (770 × 570 × 2 mm);
- 1 stainless steel plate that serves as the foundation for the valve box with heating system (Maq'nagua, Serra Negra, Brazil)
- 1 valve panel formed by a stainless steel plate of size of 320 × 270 × 2 mm (Maq'nagua, Serra Negra, Brazil)
- 1 heating valve box (310 × 260 × 120 mm) for the valve panel with fluid inlet and outlet with diameter of 22 mm (Maq'nagua, Serra Negra, Brazil)
- 1 backpressure valve (Tescon, 26-1700, Sorocaba, Brazil)
- 1 backpressure valve (Tescon, 44-2200, Sorocaba, Brazil)
- 1 backpressure valve (Tescon, 44-1800, Sorocaba, Brazil)
- 1 air pressure regulator (Norcren, R07-100-RNKA, São Paulo, Brazil)
- 1 safety valve (Swagelok, SS-4R3AS, São Paulo, Brazil)
- Tube adapter, tube OD 1/8" e NPT female 1/4" (Swagelok, SS-400-6-2, São Paulo, Brazil)
- 1 air-driven $CO_2$ pump (Maximator, M-111L, Nordhausen, Germany)
- Tube 1/8" OD 0.89 mm (Fopil, A.I.ASTMA269TP316S, Campinas, Brazil)
- Tube adapter - Union Tee Connector, tube OD 1/8" with ferrule (Fopil, ASTMA276TP316, Campinas, Brazil)
- Tube adapter - Straight Union, tube OD 1/8" with ferrule ASTM A276TP316 (Fopil, A.I.ASTMA269TP316S, Campinas, Brazil)
- Tube adapter - tube OD 1/8" e NPT Male 1/4" ASTM A276TP316 (Fopil, A.I.ASTMA269TP316S, Campinas, Brazil)
- Tube adapter - union tee male branch NPT 1/4" (Fopil, ASTMA276TP316, Campinas, Brazil)
- Cylinder outlet connection (Hoke 7115F4Y, Spartamburg, EUA)
- Blocking valve (Autoclave Engineers, 10V2071, PA, USA)
- Micrometering valve (Autoclave Engineers, 10VRMM2812, PA, USA)
- Particulate filter (Swagelok, séria F, São Paulo, Brazil)
- 2 pressure gauges - 100 bar (WIKA, EN8371-1, Klingenberg, Germany)
- 2 pressure gauges - 600 bar (WIKA, EN8371-1, Klingenberg, Germany)
- 1 pressure gauge - 400 bar (WIKA, EN8371-1, Klingenberg, Germany)
- Insulation sheets - 13 mm (Epex, Vidoflex M2, Blumenau, Brazil)
- 1 temperature indicator 173.2 K — 473.0 K (Pyrotecautomação, T4WM-TP100, Campinas, Brazil)
- 3 thermocouples - 50 mm × 1.2 m (Pyrotecautomação, PT 100, Campinas, Brazil)
- 2 thermocouples - 150 mm × 1.2 m (Pyrotecautomação, PT 100, Campinas, Brazil)
- 1 separator with heating jacket, 90 mL;
- 1 separator with cooling jacket, 90 mL
- 1 extractor with heating jacket, 100 mL;
- 1 ramp clamp (Keck SK, Max. tubing o.d. 1/4 in. / 6 mm, Munich, Germany)
- 2 ball valve (Japi, globo, Jundiaí, Brazil)

- Flowmeter (Cole Parmer, PMR1, IL, USA)
- Flow totalizer (Itrón, G25, Americana, Brazil)
- Silicone hose (Sinergia, 200 × 200, Campinas, Brazil)
- Insulation tube 1/8" (Armaflex, AFM-10, Campinas, Brazil)
- Insulation tube Ø - 22 mm (Isolan, Campinas, Brazil)
- 2 serpentine tube 1/8";
- 1 serpentine hose;
- 1 steel plate (420 × 350 × 2 mm);
- 2 hose npt adapters (female 22 mm) 1/2" (Amanco, Campinas, Brazil)
- 2 Hose Tee connectors 1/2" (Amanco, Campinas, Brazil)
- 1 extractor support structure (Autic, Campinas, Brazil)
- 4 separators support structures (Autic, Campinas, Brazil)
- 1 aluminum plate 110 x 270 x 5 mm (Autic, Campinas, Brazil)
- 1 heating bath (1500 W) 243 K — 373 K (Thermo Haake, C10, Eindhoven, Holland)
- 1 cooling bath (2000 W) 273 K — 368 K (Thermo Haake, DC30/DL30, Eindhoven, Holland)

Each topic presented in the SOP is numbered according to the sequence of the unit operational steps, and may be followed by the underlined word "Caution" with the explanation of what should be checked.

**Standard procedure to turn on and operate the SFE-0.1L unit with the secondary extraction line:**

26) Turn on the air conditioning of the extraction room for approximately 10 minutes before starting work on the unit to operate at constant room temperature; record this value;

27) Record the value of the flow totalizer (Figure 3B);

28) Connect the power cables of the temperature indicator, thermostatic bath and heating bath in voltage and amperage indicated in the plugs (Figures 3B and 4B);

29) Record the temperature of the temperature indicator (Figure 4B);

30) Release the pressure of the compressed air to the regulator (Figure 3B);

31) Close the blocking valve V1 (Figure 4B);

32) Open the $CO_2$ cylinder valve and the ball valve of the cylinder connector that carries the $CO_2$ to the tubes (Figure 5B);

33) Close the blocking valve V3 (Figure 6B);

34) Turn on the thermostatic bath until it reaches operating temperature. The maximum working temperature must be 268 K and the minimum 255 K. The thermostatic bath takes approximately 45-60 minutes to reach the temperature;

35) Disconnect the 1/8" tube of the hose t connector connected to the upper part of the extractor with a 55 mm wrench with a movement of 90° counterclockwise; after this rotation no longer use the wrench but finish with the hands (Figure 7B). Caution: If is not possible to finish opening using the hands the thread was damaged; send the cover and the thread for repair before it is no longer possible to open it using a 55 mm wrench. To open the unit cell, it must be in internal pressure conditions similar to the environment to prevent damage to their threads. The covers are made of the same material as the threads, and they could be caught into the thread if torque were applied with a key while the cell is pressurized. At the slightest sign of trouble during the opening, send the parts for repair;

36) Place the desired raw material inside the extractor;

37) Place the Teflon gasket into the cell and then screw the cover with your hands until the point where it is necessary to use the wrench. Place the center output of the hose tee connector that connects the cover to the tubing and the thermocouple with a 45 ° angle relative to the tube that connects the 1/8" tube (Figure 7B). Exert torque on the cover clockwise with a 55 mm wrench until the connection aligns with the 1/8" tubing and screw the tubing into the hose's t connector;

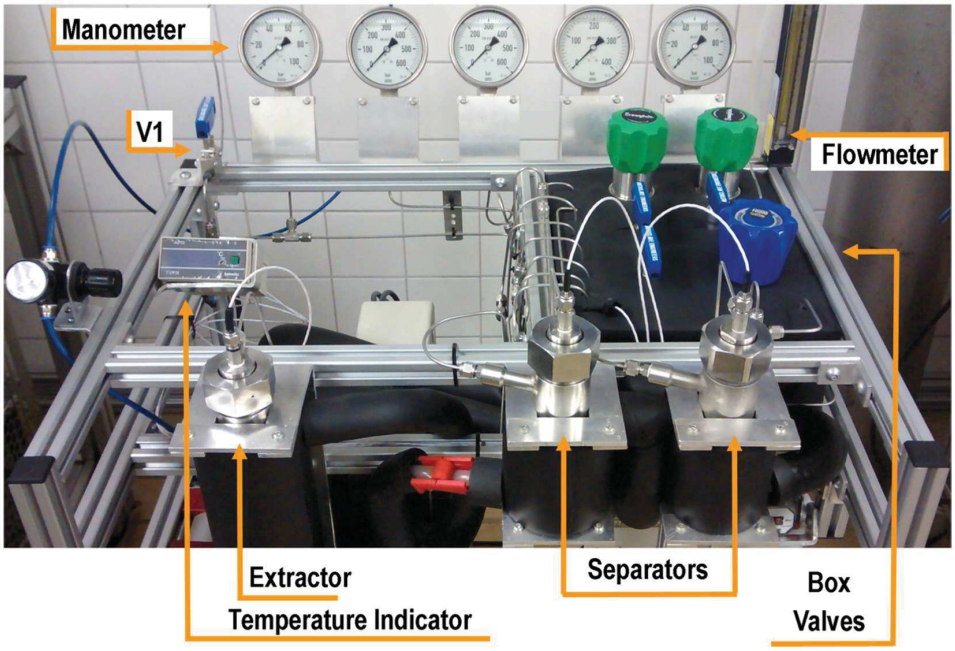



**Figure 3B**. Unit SFE-0.1L, front view.

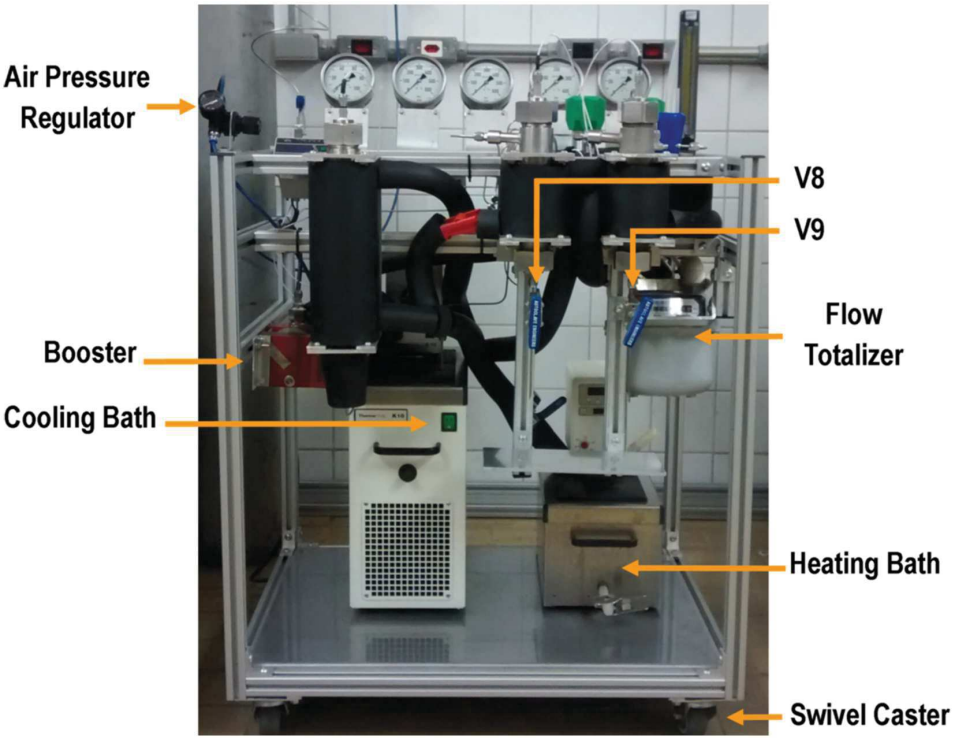



**Figure 4B**. Unit SFE-0.1L, perspective view.

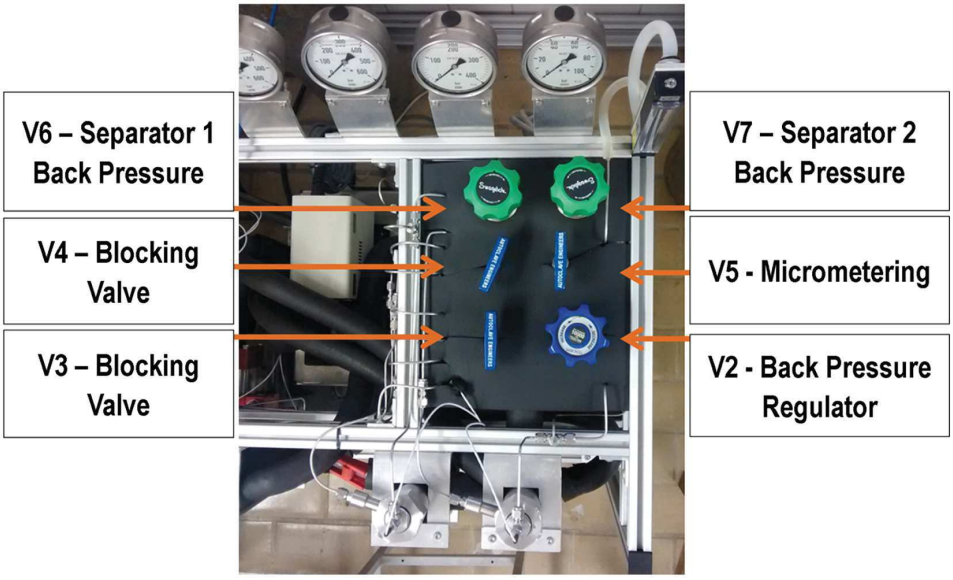



**Figure 5B**. Box valves.

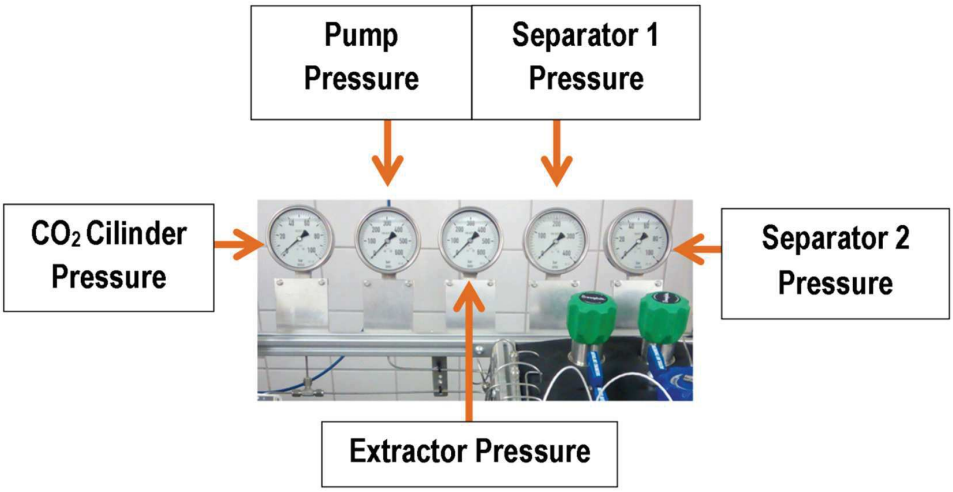



**Figure 6B**. Manometers.

38) Open the blocking valve V1; the cylinder manometer pressure should reach 60 bar (Figure 8B), in case the cylinder is full (only use the cylinder with pressure between 60 and 50 bar);

39) Before turning on the heating bath (Figure 3B), the water level should be checked. Additionally, observe if the water is clean (Figure 9B); because the jacket is made of copper, the fluid should be periodically changed to avoid rusting of the thermocouple, which could affect the temperature control. Indeed, some dirty particles could damage the pump of this component. Caution: The fluid level of the heating bath should be higher than the black plastic connected to the tube and should always be lower than the cover of this component (Figure 9B). If the fluid level is not between the indicated positions, the bath will turn off automatically and the fluid will return to the valve panel, causing overflow. To avoid this inconvenience, two ball valves at the base of the bath pump output (Figure 10B) and a type of hose valve with an open body were installed;

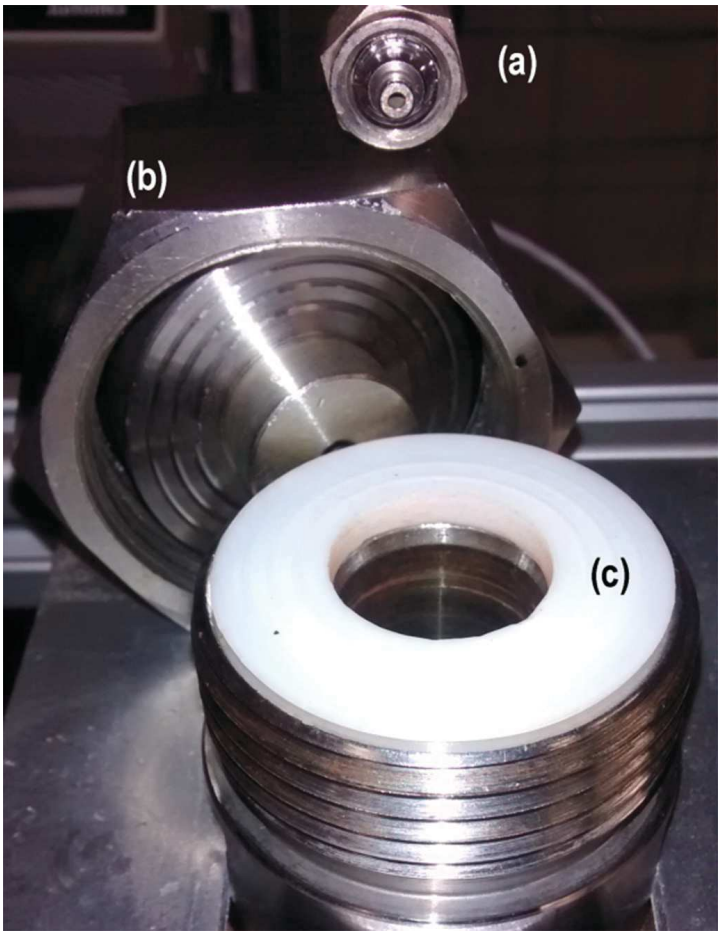



**Figure 7B**. Extractor components, (a) $CO_2$ inlet 1/8 tube; (b) Extractor - 55 mm; and (c) Teflon.

40) Turn on the heating bath. (This heating bath interchanges thermal energy with the extractor jacket and with the valve panel). Because of the specifications of the backpressure valves, the maximum temperature of the heating bath should be 353 K. Check that the bath temperature reaches the desired temperature in the extractor. If necessary during the extraction, regulate the bath temperature to keep the temperature of the thermocouple at the extractor outlet constant;

41) Then, pressurize the tube that connects the air-driven $CO_2$ pump to the valve V3. Slowly release the air pressure to the air-driven $CO_2$ pump, which should begin to move its piston at 2.5 bar of pressure with the pump regulator gauge (Figure 3B). Because the backpressure valve is still fully open, the pressure should not rise in the second manometer and the flow corresponding to the excess pressure should be recirculated to the tubing before the air-driven $CO_2$. At this point, the backpressure valve should start to slowly close, and the pressure in the second manometer will rise. The air-driven $CO_2$ pump should begin to pressurize (pump) slowly and then more pressure should be liberated from the compressed air regulator until the desired pressure is reached and the $CO_2$ is recycled by the backpressure valve. (Caution: the air-driven $CO_2$ pump should work constantly to avoid pressure changes in the compressed air that cause changes in the extractor pressure. Thus, after the initial adjustment, the equipment should work constantly until the end of the extraction process without the need for controlling the pressure of the compressed air or the back pressure, Figure 6B);

42) Once the pump has been working constantly for some seconds, recycling the flow corresponding to the excess pressure after the pump, the blocking valve V4 should be closed (Figure 6B). Then, slowly open the blocking valve (Figure 6B) until the pressures in the extraction and pump manometers are the same. Caution: Do not open the blocking valve V3 suddenly; if this happens, the pressure after the air-driven CO2 pump will fall sharply, and it will pump unevenly, which may cause undesired operation;

43) Count 20 minutes of static time;

**Figure 8B**. CO2 ball valve.

44) The micrometering valve V5 must be closed carefully. Do not close it totally and damage its needle. Caution: The micrometering valve should never be completely closed; for this reason, a blocking valve is installed in the same line for the sole purpose of performing this function. In this way, when the blocking valve is opened, the micrometering valve V5 is already in the working position and only requires an initial adjustment to maintain the flow rate;

45) After the static time and with the micrometering valve already adjusted to control the flow (this point corresponds around 45° of rotation before the flow is blocking; never completely close the valve to avoid damaging the needle), release the blocking valve V3;

46) Record the beginning of the extraction time;

47) At this time, the flow adjustment should be performed only through the micrometering valve to keep it constant during the entire process. Caution: A few seconds are spent before observing some change in the flowmeter caused by a position change in the micrometering valve. To avoid damage to the needle, it is necessary to wait for the response in the flowmeter before again adjusting the valve V5 (Figure 6B). Then, wait for approximately 15 seconds to evaluate if the flow in the flowmeter is constant.

## SFE-0.1L unit with secondary extraction line shutdown procedure:

18) Once the extraction time is finished, close the blocking valve V4 (Figure 6B);

19) Close the compressed air feeding (Figure 3B);

20) Close the blocking valve V3 (Figure 6B);

21) Close the $CO_2$ cylinder valve (Figure 5B);

22) Turn off the thermostatic bath (Figure 3B);

23) Record the flow totalizer value (Figure 3B);

24) The pressure should fall in the extractor when the air-driven $CO_2$ pump is stopped. Then carefully open the valves V4 and V5, trying to maintain a constant flow rate to depressurize the system;

25) Record the flow totalizer value corresponding to the end of the depressurization process;

26) Open the 1/8" tubing that connects to the hose t connector. The pressure will fall to the ambient pressure. With the extractor still warm, open the top cover and remove the raw material;

27) Turn off the heating bath and immediately close both blocking valves at the pump outlet (Figure 10B) to avoid heating fluid reflux;

28) Perform the cleaning procedure describe in the SFE-0.1L Unit Standard Cleaning Procedure;

29) Disconnect the power cables of the temperature indicator, thermostatic bath and heating bath.

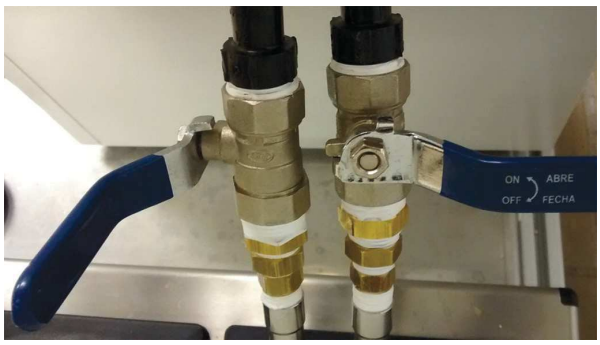



**Figure 9B**. Ball valve for water control.

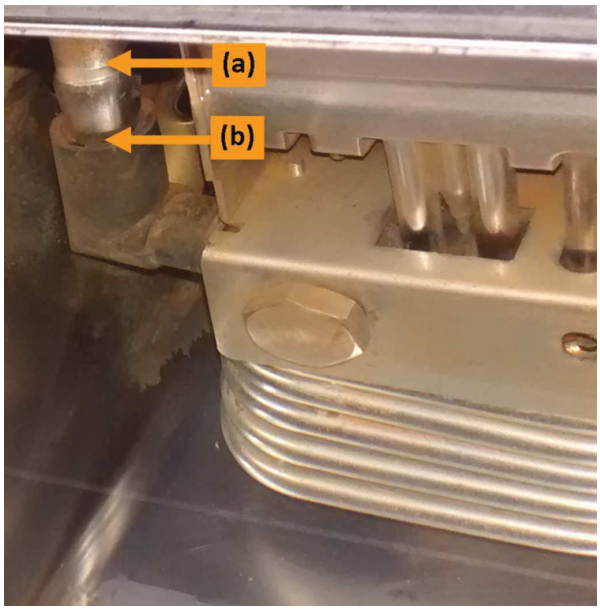



**Figure 10B**. Bath level. (a) Full mark, do not overfill (b) Add mark, add water when level is at or below this mark.

**Appendix C**. SFE-0.1L Unit Standard Cleaning Procedure (SCP). Campinas-SP, July of 2015

## SFE-0.1L Unit Standard Cleaning Procedure

A connection that uses the pressure of compressed air (6-12 bar) to clean the system was developed. This connection consists of a 1/8" tube 0.04 m in length. In that tube, one end has a connector OD-OD, and the other end has a straight connector OD-NPT that is threaded to a pneumatic connection entrance with a polyurethane tube.

## Unit Standard Cleaning Procedure

1) Open the extractor cover and disconnect the 1/8" tube with a wrench and then with a 55 mm wrench, turning it counterclockwise;
2) Remove the raw material;
3) Open the bottom cover with the same wrench as in step 1) but turning it clockwise;
4) Clean the extractor covers and the Teflon gaskets;
5) Close the extractor cover and connect the 1/8" tube to the extractor outlet;
6) Fill the extractor with a solvent that can dissolve the extract; it can be ethanol or some other solvent, depending on the extracted raw material;
7) Turn the cleaning connection (Figure 1C) at the upper extractor cover;
8) Put a collector flask at the end of the extraction line;
9) Connect the compressed air tube to the cleaning connection;
10) Pressurize the extractor with compressed air to force the flow of solvent. During the procedure is recommended that valves are open and closed for further cleaning;
11) Repeat this process until all extraction residues from the tubes and valves are totally removed;
12) After the removal of all cleaning solvent, the compressed air line should be still working for around two minutes to guarantee the removal of the remaining solvent;
13) Close the compressed air line;
14) Disconnect the cleaning connection and connect the 1/8" tube.

This procedure should be performed immediately after extraction has been performed. Thorough cleaning of pipes and valves of the equipment through this procedure ensures that no fouling is formed in the pipes and valves, avoiding clogging and reducing experimental variations.

**Figure 1C**. Cleaning Connection.

**Appendix D**. Micrometering Valve Standard Operating Procedures (SOPs). Campinas-SP, July of 2015

## Micrometering Valve Standard Operating Procedure

The cleaning procedure for the micrometering valve of the SFE-0.1L unit should be performed whenever this component is not responding correctly when it is operated, probably because there is some clogging. The micrometering valve is responsible for controlling the flow during the process; therefore, the lack of periodic maintenance could cause considerable variations in the experimental results.

## Micrometering Valve Standard Cleaning Procedure:

1. Remove the valve panel to provide access to the micrometering valve;
2. Fix the connections with a monkey wrench and disconnect unions and crosses with a wrench (1/2") to release the tubes that enter into the valve panel;
3. Attach the valve panel to the table with a bench vise as shown in Figure 1D to perform the procedure quickly;
4. Before removing the valve, fully open the blue lever to avoid any damage to the needle during the procedure;

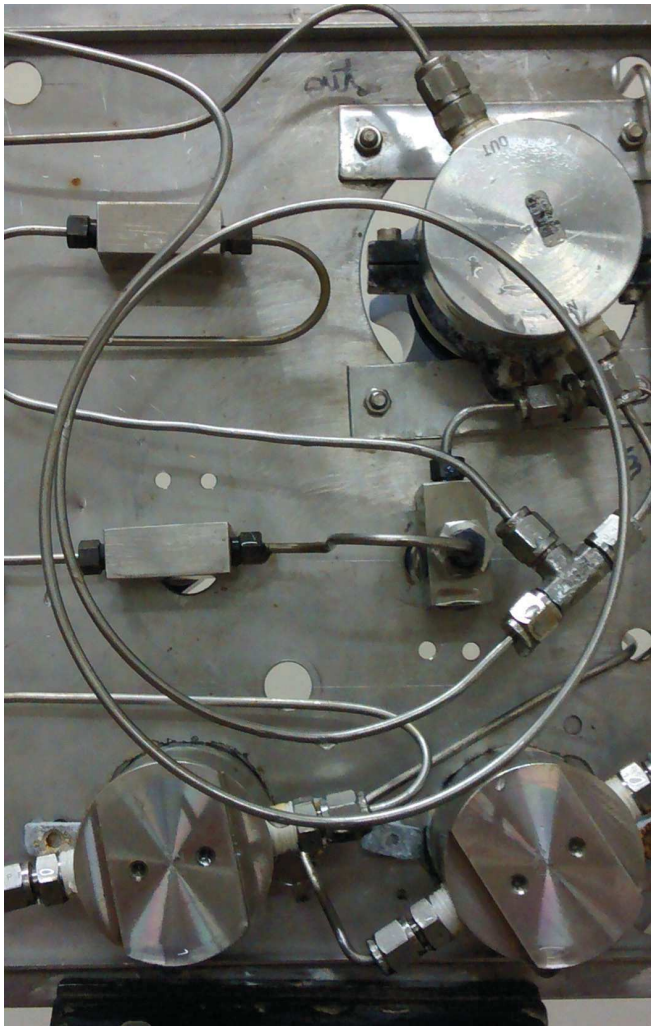


**Figure 1D**. Panel valves.

5. With an Allen wrench, remove the blue lever and the two position indicators;
6. Unscrew the valve support with a screwdriver and remove the valve near the lower part of the panel, as shown in Figure 2D;


**Figure 2D**. Micrometering valve.

7. Position the valve body with a monkey wrench or use the bench vise;
8. With a 5/8" wrench, unscrew the upper part from the lower part of the valve, as shown in Figure 3D, being careful not to miss any internal parts during the procedure. Caution: The micrometering pieces are of great precision and dropping it could damage its operation permanently;

**Figure 3D**. Opening the valve components.

9. Clean all the pieces, including the whole valve, with enough ethanol to remove any fragments that are trapped in the line at this point. Sample fragments or even valve fragments could damage the valve and cause leaking. Clean and dry all components before reassembling the valve;
10. Fit the components in the sequence shown in Figure 4D and squeeze with sufficient force to prevent leakage;


**Figure 4D**. Valve components.

11. Install the valve in the valve panel;
12. Connect the inlet and outlet tubes;
13. Install the valve panel in the unit;

Connect all 1/8” tubes that were disconnected previously to perform the valve maintenance.

*CAPÍTULO 4*

***FRACTIONATION OF ANNATTO EXTRACTS***

# FRACTIONATION OF ANNATTO EXTRACTS WITH CARBON DIOXIDE USING A HOME-MADE EQUIPMENT

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# Fractionation of Annatto Extracts with Carbon Dioxide Using a Home-Made Equipment

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**Abstract** Extracts obtained from whole annatto seeds were fractionated with supercritical carbon dioxide using a home-made experimental apparatus. The crude extracts, obtained in a 100 mL extractor at 313 K and 20 MPa were fractionated in two separator vessels, i.e., S-1 operating at 312 K and 9 MPa, and S-2 operating at 282 K and 4 MPa. Chemical composition of the resulted products was evaluated in terms of a qualitative approach using thin-layer chromatography. High-pressure phase behavior experiments were applied to the fractionated extracts using a synthetic-visual apparatus. Results indicate that annatto extracts were successfully fractionated in terms of visual aspects and differences in chemical profile. Chemical composition suggest that annatto extracts present relevant profile of terpenes and phenolics. The extracts presented similar phase behavior with the occurrence of vapor–liquid-equilibria, and vapor–liquid–liquid phase behavior for the studied compositions.

**Keywords** Supercritical fractionation, Annatto, High-pressure phase behavior, Phenolic compounds

## 1. Introduction

Annatto (*Bixa orellana* L.) plays important economic and cultural roles as a natural additive colorant in food industry. Brazil is one of the largest producers and exporters of bixin, the pigment extracted from whole seeds [14]. Considering the necessity of natural substances for the industries, there is a recent demand for nutraceuticals and health-promoting ingredients from annatto seeds related to the quality of extracts. Supercritical fluid extraction (SFE) is an option to obtain high-value products with health-promoting properties [5]. Most of recent studies on annatto are focused on improving environmentally friendly processes for bixin extraction, for instance: microwave-assisted extraction [21], supercritical fluid extraction [1], pressurized liquid extraction [3], and ultrasound-assisted extraction [26]. Furthermore there are reports focusing on the potential application of annatto as a healthy alternative to synthetic dyes in formulations of bread [20], chicken [4] and cheddar cheese [22].

Some kinds of compounds from extracts obtained via SFE may present high cost and often are present in low concentration, which reduces its value for marketing as crude extract [11]. The separation of these extracts for obtaining of new products with distinct compositions is a way to improve the quality of extracts in terms of the high concentration of the compounds of interest [25, 7]. Fractionation of natural extracts with supercritical fluids using the combination of separators results in the acquisition of high-quality products with increased purity and differentiation of bioactive compounds [23].

One of the difficulties in the extract fractionation is related to the solubility, i.e., the ability to separate volatile oils from other compounds that $CO_2$ can dissolve [12]. The main factors that interfere in the separation process are pressure and temperature. At low temperatures (-5 up to +5 °C) waxes are insoluble in $CO_2$ [13].

The knowledge of the phase equilibrium behavior of the volatile and nonvolatile fractions of foods is of crucial importance for many applications in the food industry [15]. In the case of annatto extracts it can provide theoretical data that can help the selection of process conditions. However, there are very few investigations reporting the bioactive profile of annatto extracts obtained from supercritical fluid extraction [2] and their high-pressure phase behavior [16, 17].

In this context, the goal of this work was to propose a new process for the fractionation of annatto crude extract using supercritical carbon dioxide as solvent. The phase behavior of the fractionated extracts was studied using a high-pressure equilibrium cell at three levels of temperature. In addition, the chemical profile of these products was determined using thin-layer chromatography (TLC).

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## 2. Material and Methods

### 2.1. Raw Material

Annatto variety Piave was obtained from IAC (Agronomic Institute of Campinas), Department of Agriculture and Supply from the State of São Paulo, Brazil.

### 2.2. Supercritical Fluid Extraction

Extraction of whole annatto seeds and subsequent fractionation of crude extracts were carried out in the home-made SFE-0.1L unit (Figure 1), built and validated elsewhere [9]. This unit is composed by two parts: the first is constituted of a 100 mL extractor and the second part is composed of two 90 mL separators (S-1 and S-2). Whole annatto seeds were loaded into the extractor and the extracts were collected in the two separators. Carbon dioxide, $CO_2$ (99% purity, White Martins, Campinas, Brazil) was used as solvent and SFE assays were performed at 313 K and 20 MPa at the solvent/feed ratio (or S/F) of 21. These conditions were selected because of high extract yields obtained previously [1, 2].

After SFE procedure, the extractor tubing was connected directly to the separators, whose pressure was maintained at 9 MPa in the separator S-1, and 4 MPa in the S-2 separator. The first separator has a heating jacket to maintain the temperature of 312 K and the second separator has a cooling jacket to maintain the temperature at 282 K. The pressurization prevents losses of extracts inside the second separator or mixing of extract from the first separator.

The fractionation procedure started by pressurization of the extractor until the pressure equalized that of the first separator. Afterwards the S-1 separator was depressurized until its pressure equalize to that from the S-2 separator [9]. Back pressure valve was used to control the outlet flow of solvent following steps of 10° twist paused for approximately 10 seconds.

After equalization of pressures between the separator vessels, the fractionated extracts were collected and the last back pressure valve was opened, performing the procedure previously mentioned.

### 2.3. Thin-layer Chromatography

Silica gel plates with aluminum backs (Alugram®, Xtra SIL G, Macherey-Nagel, Germany) were used as stationary phase. The mobile phase was composed of chloroform (Merck, Darmstadt, Germany), ethanol (Chemco, Hortolandia, Brazil) and glacial acetic acid (Synth, Diadema, Brazil), in the proportion of 95/05/01, v/v/v [24].

To facilitate the identification of the compounds the following standards were used: ar-turmerone, trans-caryophyllene, camphor, carvacrol, α –humullene, limonene, r-carvon, terpineol (mixed isomers, principally α, approximately 95%), p-cymene (99%) and α-bisabolol (>85%), obtained from Sigma-Aldrich (Darmstadt, Germany). Bixin standard was obtained with exhaustive extraction with acetone, according to the procedure described elsewhere [9].

The bands of compounds generated by the constituents that could not be detected in the visible region were visualized using a UV (Multiband UV – 254-366 nm, UVGL-58, Mineralight® Lamp, Upland, CA, EUA) equipped with a cabinet (UVP-Chromato-VUE, CC-10, Upland, CA, EUA) for short wavelength (254 nm) and long wavelength (366 nm) visualization. The phenolics and volatiles were detected using the sulfuric p-anisaldehyde spray reagent, according to the formulation proposed by Hamilton & Hamilton [8] and the sulfuric vanillin spray reagent, according to the formulation proposed by Krishnaswamy [10]. The p-anisaldehyde and vanillin used in this work were purchased from Sigma-Aldrich (Darmstadt, Germany) and Synth (Diadema, Brazil), respectively. The sulfuric acid used in this work was obtained from Exodo (Hortolandia, Brazil).

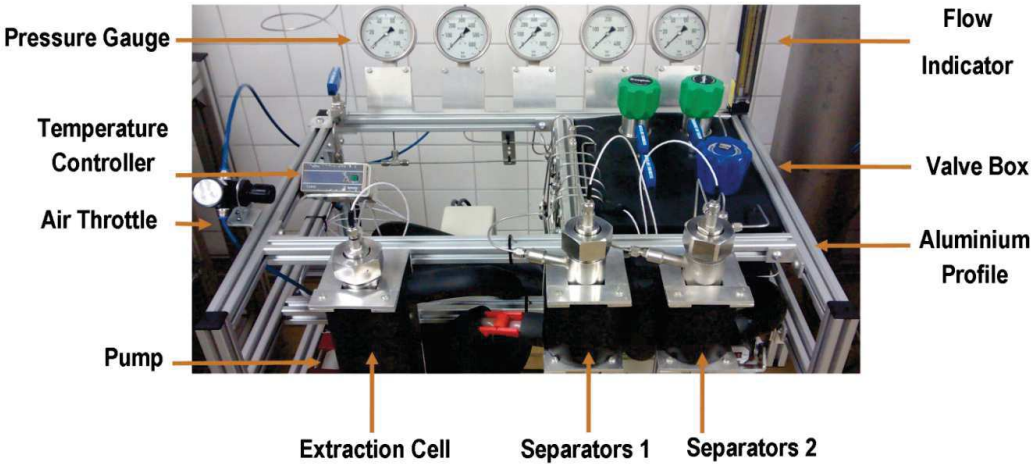


**Figure 1.** Home-made SFE-0.1L unit

Approximately 10 μL of the samples were spotted on the thin-layer chromatography (TLC) plates with the aid of chromatographic syringe (Hamilton, Darmstadt, Germany) with approximately 1 cm distance from each band. Afterwards the plates were developed into glass chambers by elution in mobile phase. After elution, the compounds of interest were detected by spraying the plates using the sulfuric p-anisaldehyde, or the sulfuric vanillin, reagents. After detection with p-anisaldehyde the plates were subjected to thermal activation using an oven (Tecnal, TE-385-1, Piracicaba, São Paulo) at 373 K for 5 minutes.

### 2.4. High-Pressure Phase Equilibrium

High-pressure phase behaviour experiments of annatto fractionated extracts in the presence of compressed $CO_2$ were performed adopting the synthetic method with phase transition visualization using a high-pressure variable-volume cell, from which apparatus was built [15] and validated [16].

Three levels of temperature (303, 313 and 323 K) were used to perform high-pressure phase equilibrium assays with a minimum of three replicates. Due to the poor availability of sample, the extracts were loaded into the cell with the aid of a 10 μL chromatographic syringe (Hamilton, Sigma-Aldrich, Darmstadt, Germany). The overall composition of $CO_2$ expressed in terms of mass fraction ($\omega_1$) varied from 0.92 to 0.99.

## 3. Results and Discussion

### 3.1. Extraction and Fractionation

Crude annatto extract obtained at S/F=21 yielded 2% from whole seeds. This yield was slightly lower than 3.8% obtained previously at S/F=37 [9] because high values of S/F imply exhaustion of the raw material, and consequently, high extract yields. Besides this, lower yields may be associated with loss of extract inside the S-1 Figure 2C) and S-2 (Figure 2D) separators because the extracts are collected directly with the aid of the micrometering valve, which reduces the course of the extract until the flask collector.

The fractionated extract collected from the S-1 separator (Figures 2A and 2C) corresponds to 58% from crude annatto extract, while the extract collected from S-2 separator (Figures 2B and 2D) corresponds to 41% from the crude annatto extract.

Depressurization prevented loss of extract in the pipeline located after the second separator. Cooling of the second separator is another factor that allowed complete separation of extract from solvent, because of differences in densities between solvent and extracts.

Obtaining of $CO_2$ with low residual content of extract is a determining factor for the recycling operation of this solvent, since the separation procedure for complete $CO_2$ removal may carry a part of the extract to the recycling line, which causes loss of extract and further damages the recycling equipment.

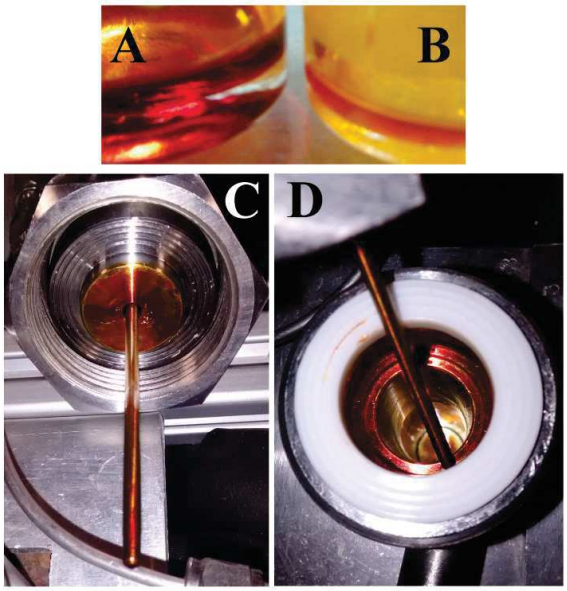


**Figure 2.** Annatto fractionated extracts obtained from S-1 (A) and S-2 (B, C and D) separators

### 3.2. Thin-Layer Chromatography

The fingerprints of fractionated annatto extracts differ from those reported elsewhere for crude annatto extracts obtained with supercritical $CO_2$ and developed with n-hexane/ethyl acetate/formic acid (v/v/v) mobile phase [2, 9].

Both p-anisaldehyde and sulfuric vanillin spray reagent are universal reagents for natural products, making color differentiation possible, i.e., they are reagents used to detect antioxidants, steroids, prostaglandins, carbohydrates, phenols, glycosides, sapogenins, volatile oil components (or terpenes), antibiotics and mycotoxins, depending on the preparation of sample, type of stationary phase and mobile phase formulations [24, 6].

The manner of detection of sulfuric p-anisaldehyde spray reagent differs from sulfuric vanillin by the need of thermal activation after spraying. After thermal activation with this spray reagent, only annatto fractionated extracts showed compounds that could be visualized in UV at 240 nm and 360 nm (Figure 3A). The formulation of vanillin-sulfuric acid spray reagent used in this work it is a highly destructive reagent, which is applied along with thermal activation, resulting in partial destruction of TLC plate, as can be observed in Figure 3B.

Sulfuric acid is an oxidant and its reaction with terpenes in the presence of vanillin results in the formation of purple spots, which are observed in the case of trans-caryophyllene, α-humullene, terpineol and α-bisabolol standards (Figure 3B).

After reaction between vanillin with sulfuric acid and

p–anisaldehyde coupled with thermal activation, the resultant yellow or red coloration bands are associated with phenolics, and dark-blue or purple bands are associated to volatiles, very similar to the bands of waste turmeric products detected with vanillin-sulfuric acid spray reagent [19, 18].

After spraying with the sulfuric vanillin reagent, the purple bands appeared on the extracts (Figure 3B), similarly as trans-caryophyllene, α-humullene, terpineol and α-bisabolol standards (Figure 3B) indicating the presence of volatile terpenes. The presence of pink and light-red bands are associated to phenolics (Figure 3B).

Thermal activation of fractionated extracts in the TLC plates sprayed with p-anisaldehyde reagent resulted in the slight modification of yellow coloration which turned into dark-blue (Figure 3A), similar to that reported previously for crude annatto extracts obtained with supercritical $CO_2$ [2]. Bands of fractionated annatto extracts indicate the presence of α-bisabolol, because of similarities in fingerprint and band coloration (Figures 3A and 3B).

### 3.3. High-Pressure Phase Equilibrium

The experimental measurements for the multicomponent system containing annatto extracts showed a slight variation of phase transitions at higher proportions of $CO_2$. Vapor-liquid equilibria (VLE) and vapor liquid-liquid equilibria (VLLE) phase transitions were observed, according to Table 1.

**Table 1.** Phase equilibrium data for the system $CO_2$(1)+annatto extracts(2)

| Separator 1 | | | Separator 2 | | |
|---|---|---|---|---|---|
| T (K) | P (MPa) | Phase transition | T (K) | P (MPa) | Phase transition |
| | *$\omega_1$=0,92* | | | *$\omega_1$=0.97* | |
| 303 | 7±0.1 | VLLE | 303 | 7.06±0.2 | VLLE |
| 313 | 8.6±0.2 | VLLE | 313 | 9.14±0.3 | VLLE |
| 323 | 10.2±0.5 | VLLE | 323 | 10.91±0.1 | VLLE |
| | *$\omega_1$=0.94* | | | *$\omega_1$=0.98* | |
| 303 | 6.92±0.5 | VLLE | 303 | 7.13±0.2 | VLLE |
| 313 | 9.09±0.6 | VLLE | 313 | 9.62±0.2 | VLLE |
| 323 | 10.2±0.7 | VLLE | 323 | 10.60±0 | VLLE |
| | *$\omega_1$=0.97* | | | *$\omega_1$=0.99* | |
| 303 | 7.15±0.1 | VLE | 303 | 7.10±0.1 | VLE |
| 313 | 8.04±0.8 | VLLE | 313 | 8.11±0.5 | VLLE |
| 323 | 10.8±1 | VLLE | 323 | 9.40±1.0 | VLLE |

The phase behavior for the extract obtained from the S-2 separator in the presence of supercritical carbon dioxide is visualized on Figure 4.

In the assays with higher fraction of carbon dioxide, the appearance of vapor liquid equilibria at 303 K, followed by vapor liquid-liquid equilibria at higher temperatures, is justified by the fact that annatto extracts are not pure substances. Therefore, multiple solubilities may happen, despite not getting visually detected in the apparatus used. Similar behavior was reported for the pseudobinary systems containing fractionated turmeric extracts in the presence of $CO_2$ [16].

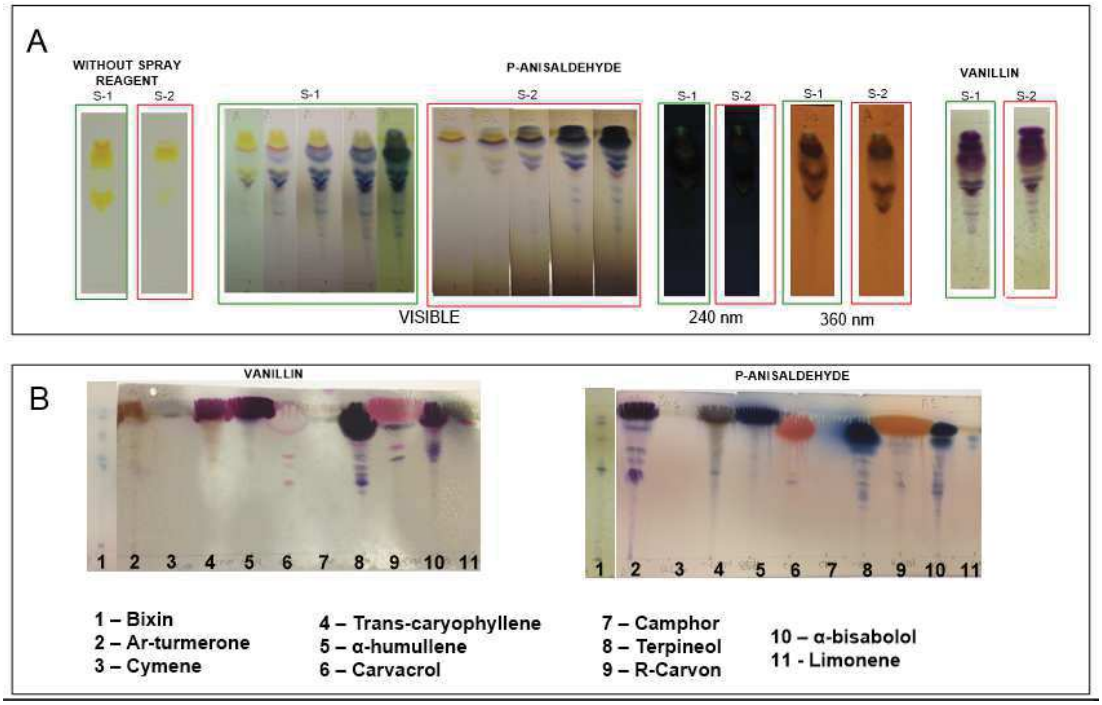


**Figure 3.** Thin-layer chromatography plates of annatto extracts collected from the S-1 and S-2 separators (A), bixin and volatiles standards

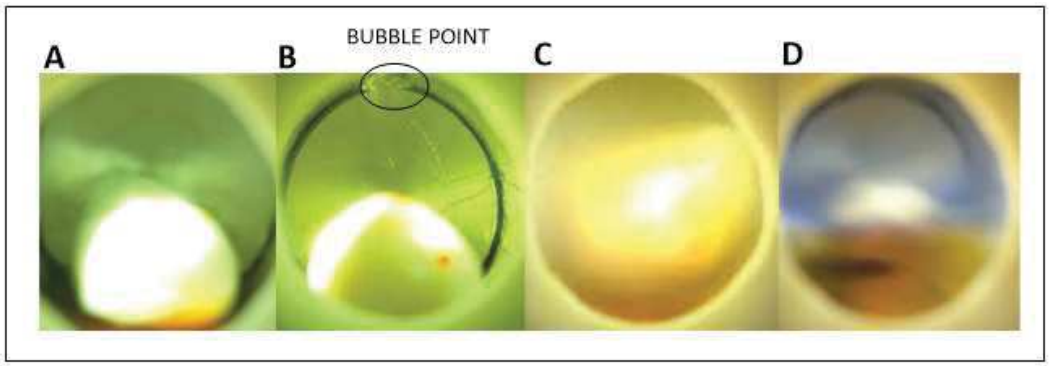


**Figure 4.** High-pressure phase behavior of fractionated annatto extract from the second separator in the presence of 99.9% of supercritical carbon dioxide at 303 K in the supercritical state (A), in the moment of vapor-liquid equilibria phase transition (B) and at 323K in the moment of vapor-liquid-liquid phase transition (C) and at pressures lower than the phase transition (D)

## 4. Conclusions

In this work, crude annatto extracts were fractionated with supercritical and compressed carbon dioxide using a home-made experimental apparatus.

Fractionation of annatto extracts resulted in two distinct products, with relevant chemical composition in terms of terpenes and phenolic constituents, which are potential health-promoting agents, that can be included in foods drugs and cosmetic products. High-pressure phase equilibrium data for the pseudobinary systems involving supercritical carbon dioxide and annatto extracts were similar and presented complex behavior with vapor-liquid and vapor liquid-liquid phase transitions because of multicomponent nature of the extracts.

## ACKNOWLEDGEMENTS

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# *CAPÍTULO 5*

## ***SUPERCRITICAL FLUID EXTRACTION ASSISTED BY PRESSING***

# DEVELOPING A SUPERCRITICAL FLUID EXTRACTION METHOD ASSISTED BY COLD PRESSING: A NOVEL EXTRACTION TECHNIQUE WITH PROMISING PERFORMANCE APPLIED TO PEQUI (*Caryocar brasiliense*)

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Artigo em processo de submissão na revista *Journal of Supercritical Fluids*

# Developing a supercritical fluid extraction method assisted by cold pressing: A novel extraction technique with promising performance applied to pequi (*Caryocar brasiliense*)

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**Abstract**

A novel technique for enhancing the performance of supercritical fluid extraction (SFE) was developed in this study. This technique integrates the cold pressed method and the SFE technique called supercritical fluid extraction assisted by pressing (SFEAP). SFEAP was compared with SFE in terms of its extraction performance of pequi, which is a native Brazilian fruit with a high lipid content. It was found that SFEAP obtained an extract mass eight times higher than that of SFE during the first minute of extraction, which corresponded to an increase of yield of 18.2 g/100g of raw material. SFEAP can be efficiently used for other raw materials with high lipid contents to decrease both the extraction time and solvent consumption.

**Keywords**: Home-made extraction unit, supercritical fractionation, Brazilian savanna, innovation and technical improvement.

## 1 INTRODUCTION

Supercritical fluid extraction (SFE) with $CO_2$ is a green technology for extracting oil from vegetables. This technique is very efficient in terms of its extraction yield, selectivity, and separation [1]. On the contrary, extraction by the cold pressed method is very fast and does not require any solvent, but cannot completely remove the oil content of the raw material, generating an extract with impurities, which may result in an extract with lower bioactivity compared to that generated by SFE [2]. To overcome the disadvantages of the cold pressed technique, as well as to decrease the solvent consumption in the SFE method, a novel extraction method was successfully developed in the current study by integrating the SFE and cold pressed methods. This new technique is called SFEAP, and its performance was evaluated by extraction from pequi (*Caryocar brasiliense*). This fruit, which is found throughout the Brazilian savanna or Brazilian Cerrado, should not be confused with pequiá-pequi (*Caryocar villosum*), a characteristic species of the Amazonian forest. Another similar species is the pequirim-pequi (*Caryocar coriaceum*), which is predominant in Northeast

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Brazilian and is a fruit that belongs to the family Caryocaraceae [3,4]. Pequi (*Caryocar brasiliense*) is the most collected fruit of the Brazilian Cerrado, has an orange yellow pulp and is well-known as tiny gold or pequi [5,6]. A comprehensive search over the past 10 years of the Science Direct database using the word *Caryocar brasiliense* (Abstract, title, and keywords) resulted in only 25 articles [7]. Of these 25 papers, 44% are on the beneficial effects of the consumption of pequi pulp and bioactive analyses, 16% are related to its tree, 16% are related to fruit processing, 8% are related to biodiesel production, and 16% are about other relevant topics, such as the aroma composition and biosensors. However, until now, no work has reported on the extraction of pequi oil via SFE.

Fig. 1 shows three types of common pequi with different colors, namely, orange, white, and yellow. In the current study, orange color pequi, which was originally from the Xingu region of Mato Grosso, was used. As shown Fig. 1, this type of pequi has a larger size and higher amount of pulp per fruit than the other pequi [8].

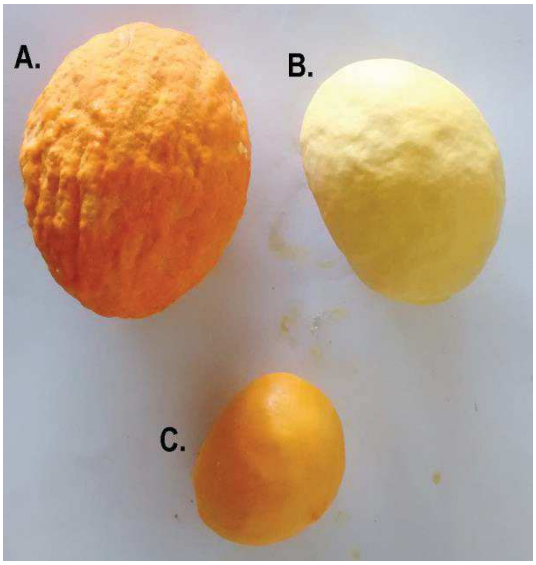



**Figure 1 –** Three types of Pequi. A. Orange color characteristic of the Xingu region of Mato Grosso. B. White color. C. Yellow color.

The traditional process of oil extraction from pequi consists of intensive cooking in water and subsequent removal of the supernatant. The main disadvantages of this extraction method are its low yield, high temperature requirement, and the fact that the oil is not filtered [9]. Extraction with supercritical fluid using $CO_2$ as a solvent represents a more advantageous method for the extraction of pequi oil due to its low temperatures (313 - 333K) [10,11]

The pulp of pequi is composed of water (48%) and oil (30%), and it is well-known to be a powerful antioxidant [12-14]. This mesocarp has 20% more riboflavin (Vitamin B2) than a chicken egg, 5.4-27 mg of total carotenoids/100 g and 45 mg vitamin C/100 g [14-16]. An analysis of the minerals present in 100 g of pequi pulp from three Brazilian states showed, on average, 72.5 mg of calcium, 1.2 mg of iron, 3.4 mg of zinc, 184.4 mg of phosphorus, 95.2 mg of magnesium, and 631 mg of potassium [17]. Pequi oil is composed of 53% oleic acid, 2.7% linoleic acid, and 39% palmitic acid and has been demonstrated to be an anti-inflammatory and antioxidant [6, 18, 19]. The dried pulp of pequi contains approximately 60% lipids [14, 20].

## 2. Material and methods

### 2.1. Raw material

Pequi was acquired in the city of Barra do Garças, MT after being collected from the Xingu region of Mato Grosso. The fruits were peeled manually using a knife (Tramontina, 21198315, Carlos Barbosa, Brazil) in a dark environment to avoid any degradation of the carotenoids and other light-sensitive biocompounds. All parts of the fruit were weighed individually using a balance (STC02, São Paulo, Brazil). Its shell was weighed and stored at a temperature of 280 K (CRM43NB, Consul, Joinville, Brazil). Its pulp was manually cut using a knife (Tramontina, 22902/007, Carlos Barbosa, Brazil) in a dark environment at 293 K. The pulp slices were frozen at 255 K and then transported to LASEFI/FEA/UNICAMP, where they were first dried in a circulation oven (Marconi, MA035 / 5, Piracicaba, Brazil) at 318 K for 24 h and then frozen at 255 K (Metalfrio, HC -4, São Paulo, Brazil). After that, the dried pulp was ground in a mini-processor-coupled mixer (Philips Walita 400 W, RI1364 / 07, Varginha, Brazil) for 50 seconds. This short grinding time was chosen to prevent agglomeration of the fine particles as well as to reduce the loss of volatile compounds [21]. To determine the particle diameter of the ground material, a sieve shaker (Bertel, N. 1868, Caieiras, Brazil) with 16-80 mesh sieves was used, and the average diameter was calculated according to the proposed equation by the American National Standard Institute [22].

The apparent density of the raw material was calculated according to Zabot et al. [23], and its actual density was measured using a helium gas pycnometer (Quantachrome Instruments, Ultrapyc 1200e, Boynton Beach, USA).

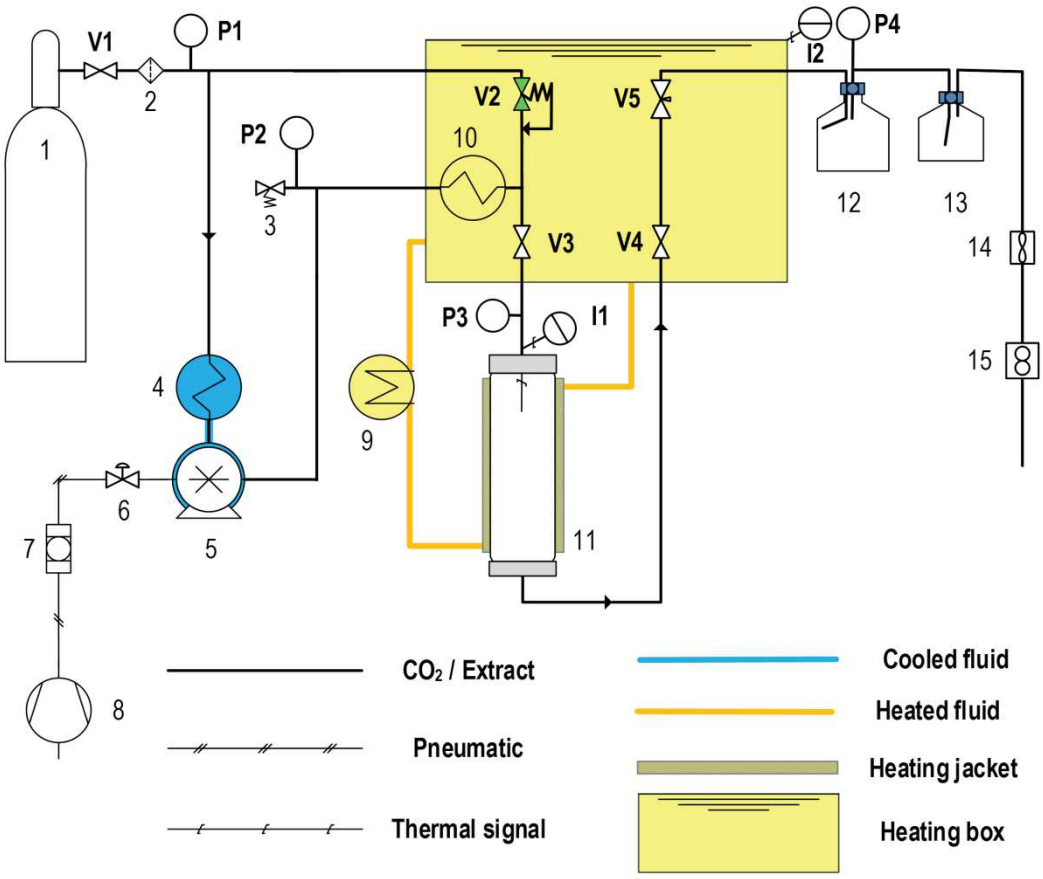
The moisture and ash contents of the material were measured according to the method proposed by AOAC international. Protein analysis was performed using the Kjeldahl semi-micro method based on a conversion factor of 6.25 [24]. Total lipid analysis was performed according to the Bligh and Dyer method using chloroform as the solvent, and its

carbohydrate content was calculated based on the difference between the total weight and aforementioned contents [25].

2.2. Supercritical fluid extraction

Extractions with supercritical fluid were performed in SFE equipment validated by Johner and Meireles [26], using two vials (250 mL and 100 mL) as separators instead of metal separators (Fig. 2). A 1/4" T-junction NPT (Fopil, A.276316, Campinas, Brazil) was attached on top of the first vial to connect it to both the manometer (WIKA, 0-0.2 MPa, Iperó, Brazil) and second vial. Three OD-NPT connectors (Fopil, ASTMA269TP316S, Campinas, Brazil) were also attached on top of the second vial and filter vial. Moreover, a silicone hose (Sinergia, 200 × 200, Campinas, Brazil) was used to connect the outlet of the last flask to the flowmeter. For the kinetic studies, only the first collection vial (100 mL) was used owing to the small amount of extract in the second collection vial.

In a typical SFE experiment, the extractor was filled with 10 grams of raw material, and the remaining volume was filled with glass beads. The temperature was measured using a thermocouple inserted inside the extractor. Extraction was performed at pressures of 20, 25, 30, 35 and 40 MPa and temperatures of 313 and 333 K, with a constant solvent per feed ratio (S/F) of 84 using $CO_2$ at 99% purity as the solvent (White Martins, Campinas, Brazil). After determining the optimum combination of the temperature and pressure, the extraction kinetics were assessed under the optimum operating condition at an S/F ratio of 352. Due to the high value of S/F, the mass of the extract collected in the second vial was measured at the end of the dynamic process and was divided evenly among the 19 extraction kinetic points.




| | | | | | |
|---|---|---|---|---|---|
| 1 | $CO_2$ reservoir | 8 | Air compressor | 15 | Flow totalizer |
| 2 | $CO_2$ filter | 9 | Heating bath | V2 | Back pressure |
| 3 | Safety valve | 10 | Serpentine tube | V5 | Micrometering valve |
| 4 | Cooling bath | 11 | Extraction cell | V(1,3,4) - Blocking valve | |
| 5 | Air-driven $CO_2$ pump | 12 | 1º Extract collecting vessel | P(1,2,3,4) - Pressure gauge | |
| 6 | Control (air flow) | 13 | 2º Extract collecting vessel | I1 - Temperature Indicator | |
| 7 | Air filter | 14 | Flowmeter | I2 - Temperature Indicator | |

**Figure 2** –The adapted SFE-0.1L apparatus [27].

### 2.3 Supercritical Fluid Extraction Assisted by Pressing

A schematic of SFEAP system is shown in Fig. 3. The pressing part was designed manually and then was assembled in the LASEFI - Laboratory of Supercritical Technology: Extraction, Fractionation and Identification of Vegetal Extracts. The pressing process of the SFEAP method was carried out inside the extractor. For this purpose, 10 grams of raw material was inserted inside the extractor, and then, the pressing piston was attached to the extractor to apply two different torques, 40 and 120 Nm. A torquemeter (Sata, ST96303SC, Sorocaba, Brazil) was used to control the force that the piston exerted on the bed. After contacting the piston with the raw material, torque was applied to the thread, and the force on

the raw material increased until the equipment clicked, indicating that the selected torque was reached. After the clicking sound was heard, the piston was immediately detached, and glass beads were inserted to fill the remaining part of the extraction column. Subsequently, SFE was performed as described in subsection 2.2.

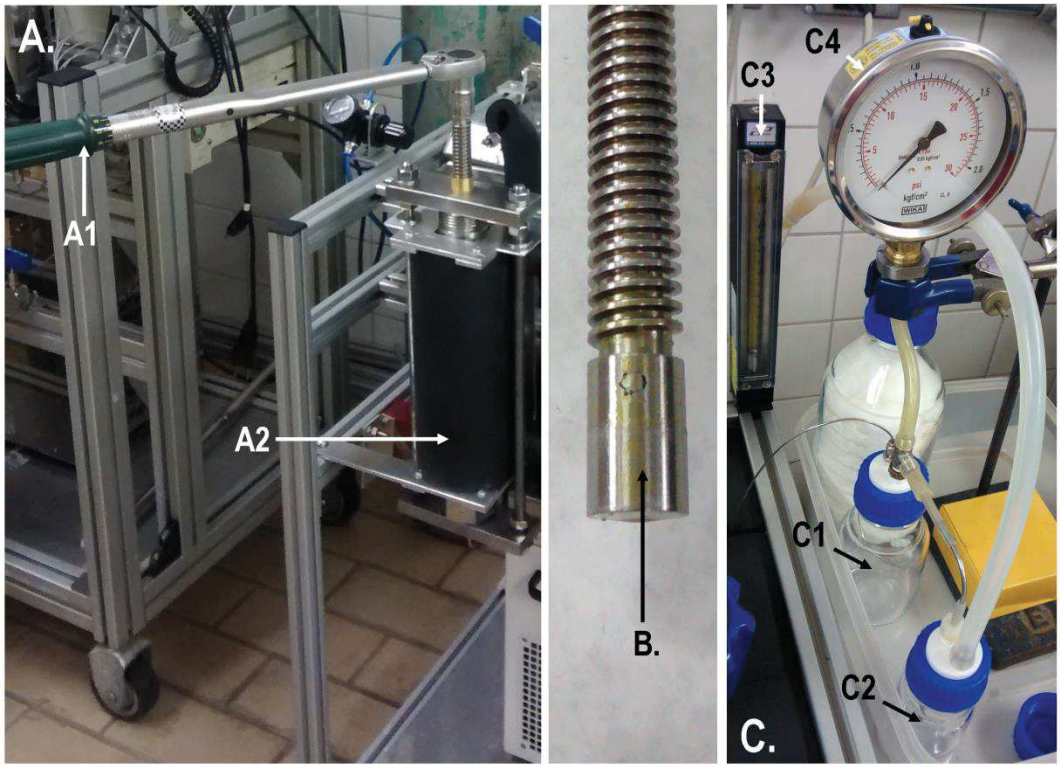



**Figura 3** – A schematic of SFEAP system, A - SFEAP system, $A_1$ - Torquimeter, $A_2$ - Extractor, B - Piston, C - Separators, $C_1$ and $C_2$ - Collectors, $C_3$ - Flowmeter, $C_4$ – Manometer.

**2.4 Oil Analysis**

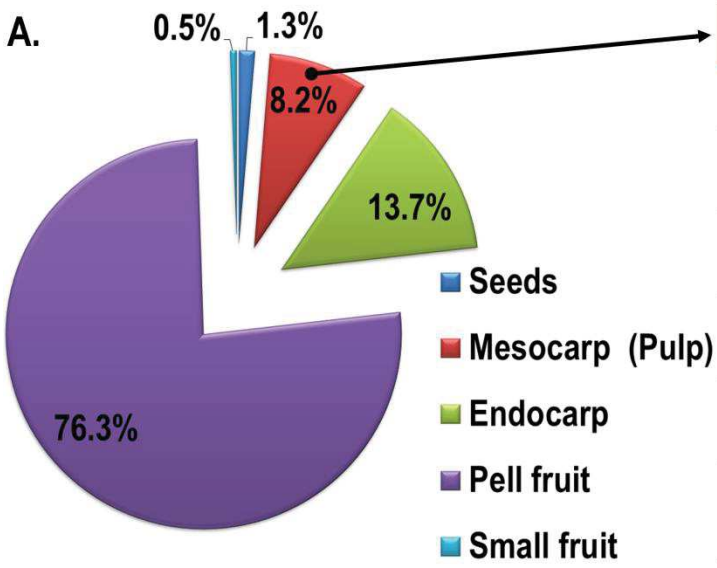
Analyzes of the fatty acid composition of the oil extracted were carried out according to the methods of AOCS (2009) [27].

**3 RESULTS AND DISCUSSION**

The actual density of the extraction bed was 1070 kg/m$^3$ (± 10), bulk density without pressing was 171 kg/m$^3$ (± 26), and bulk density after pressing at 40 Nm and 120 Nm was 253 kg/m$^3$ (± 48) and 323 kg/m$^3$ (± 23), respectively. Moreover, the density of pure $CO_2$ at 33 K and 40 MPa is 0.89 g/mL based on the Nist Chemistry Web Book [28]. The mean particle diameter of pequi was 3.4 mm (± 0.3).

Fig. 4 reveals the composition of pequi obtained in the current study. This figure gives valuable information about the amount of raw material that must be purchased to attain the desired amount of oil. The numbers presented in this figure are in line with those already

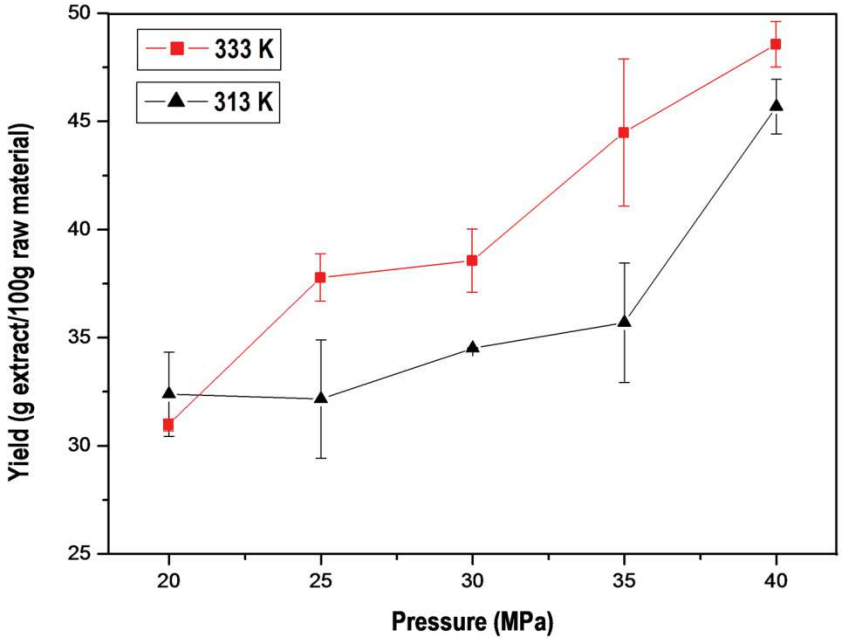
reported for the composition of pequi in the state of Minas Gerais without considering the contribution of the fruit peel. The literature reports a composition of 8% seeds, 61% endocarp, and 31% mesocarp, while the corresponding numbers in the current study were 6%, 59% and 35%, respectively [29].




| Dry Pulp | |
|---|---|
| B. | (%) |
| Water | 6.8 ±0.2 |
| Potein | 8.3 ±0.1 |
| Lipids | 57.6 ±0.8 |
| Minerals | 1.9 ±0.1 |
| Carbohydrates | 25.4* |

**Figure 4** – Compositions of pequi fruit. (A.) Graph of mass distribution of each part of Pequi fruit (B.) Table of centesimal composition of dried and ground pequi pulp (* Calculated by difference method).

Fig. 5 reveals the overall yield of SFE from pequi at 313 and 333 K and 20 to 40 MPa. As seen from this figure, the highest yield, for both isotherms, was attained at 40 MPa, followed by 35 MPa. The impact of increasing the temperature was more effective at 35 MPa and 25 MPa and increased the extraction yield by approximately 9 and 6 g extract/100 g raw material, respectively. This figure also shows that increasing the pressure from 20 to 40 MPa increased the extraction yield from 32.5 to 45 g extract/100 g raw material for the isotherm at 313 K and from 31 to 48 g extract/100 g raw material for the isotherm at 333 K.




| Density (kg/m³) | 313 K | 333 K |
|---|---|---|
| 20 MPa | 839.8 | 723.7 |
| 25 MPa | 879.5 | 786.6 |
| 30 MPa | 909.9 | 829.7 |
| 35 MPa | 934.8 | 862.9 |
| 40 MPa | 956.1 | 890.1 |

**Figure 5** – The overall yield of SFE from pequi at various pressures and temperatures during 45 min.

SFEAP using 40 Nm and 120 Nm torque was also considered in this study, and it was found that these torques gave very similar final yields, 51 (± 2) for 40 Nm and 53 (± 2) for 120 Nm. These close extraction yields together with the possible damage to the extractor by applying a torque of 120 Nm convinced us to only used 40 Nm torque for subsequent experiments. Fig. 6 shows the results of the kinetic extraction of pequi using SFE and SFEAP at 40 Nm. The pressure in the first collection vial was 0.065 MPa, while the pressure in the second collection vial was lower than the detection limit of the manometer (0.005 MPa). As shown in Figs. 6, cold pressing decisively affected the extraction yield, generating a mass of the extract during the first minute of extraction by the SFEAP technique that was approximately eight times higher than that generated by SFE. This dramatic increase in the extraction yield is due to the cold pressed step, which liberated the extracted oil from pequi. However, the final yield of both the SFEAP and SFE processes were very close, 55% for SFEAP and 53.4% for SFE. Moreover, the smaller error-bars for SFEAP indicate that the reproducibility of the data is higher than that for SFE.

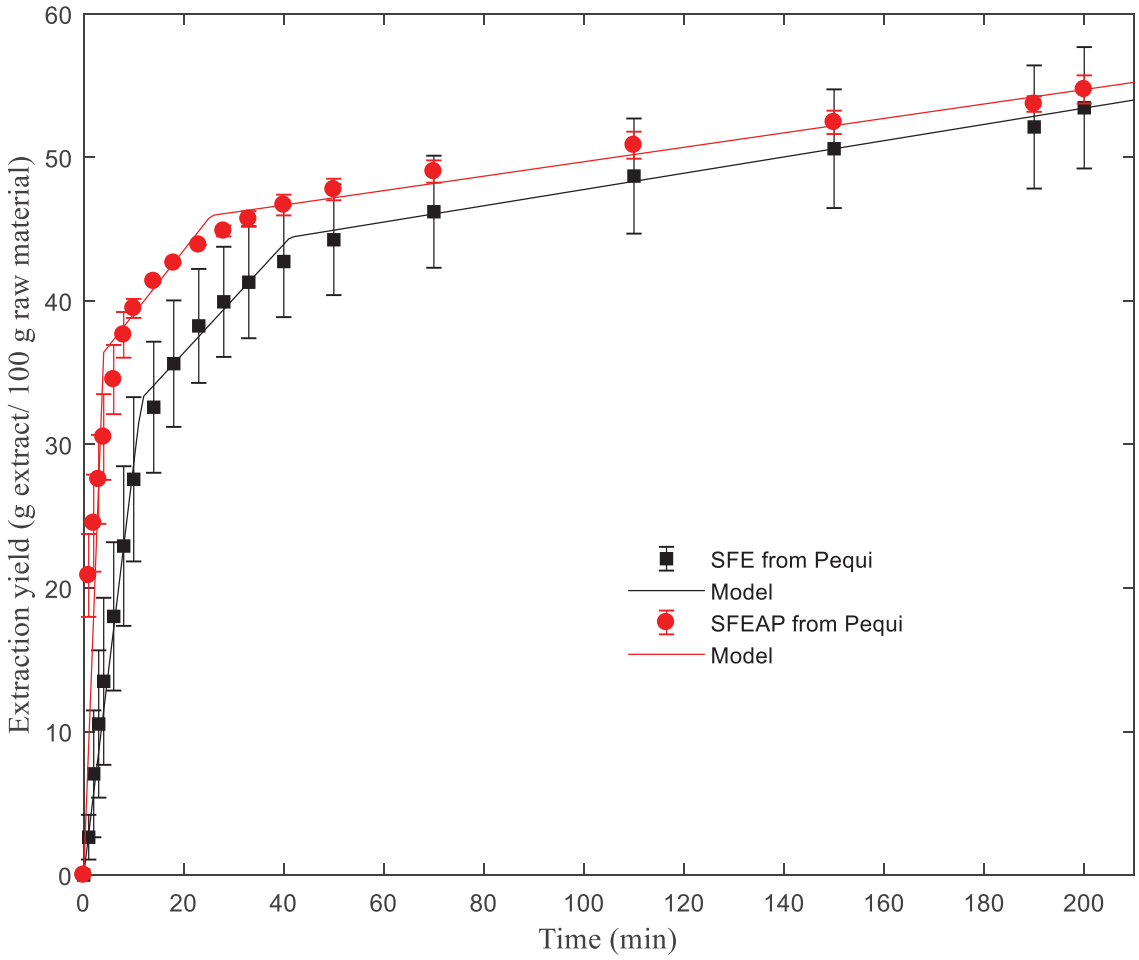



**Figure 6** – Kinetic extraction of pequi. (■)SFE - Kinetics of the extraction of the dry pequi pulp. (——) fitted model (●)SFEAP - Press assisted extraction kinetics (——) fitted model.

The extraction dynamic data were fitted to the following spline model [10] using MATLAB software, which are shown in Figure 6.

$$m_{ext} = b_1 t \qquad t < t_{CER}$$

$$m_{ext} = b_2 t + (b_1 - b_2) t_{CER} \qquad t_{CER} \leq t < t_{FER} \qquad (1)$$

$$m_{ext} = b_3 t + (b_2 - b_3) t_{FER} + (b_1 - b_2) t_{CER} \qquad t_{FER} \leq t$$

Where, $b_1$, $b_2$, and $b_3$ are the slopes of first, second, and third lines, respectively. Moreover, $t_{CER}$ is the intercept of the first and second lines, and $t_{FER}$ is the intercept of the second and third lines. Table 1 represents the constant of these three lines model for both SFE and SFEAP at 40 MPa and 333 K. Due to the availability of experimental data at various extraction time, the constants of equation (1) were obtained with high accuracy. It can be inferred from Figs. 5 that the model agreement with the experimental data is very well.

**Table 1** – Estimated data for pequi overall extraction curve.

| Constants | SFE | SFEAP |
|---|---|---|
| $b_1$ | 2.87 | 9.10 |
| $b_2$ | 0.38 | 0.44 |
| $b_3$ | 0.06 | 0.05 |
| $t_{CER}$ | 11.59 | 4.00 |
| $t_{FER}$ | 29.70 | 21.44 |

Table 2 represents the fatty acid composition of pequi extracts for the first and second collectors using SFE and SFEAP methods at 40 MPa and 333 K. The extracts obtained in the first and second collection flasks of the SFE process showed similar fatty acid profiles. The results also indicate that the SFEAP process did not influence the fatty acid composition of the extract. It was also found that the main compounds of pequi oil were unsaturated fatty acids, Omega 9, Omega 6, and saturated Palmitic.

**Table 2** - Fatty acid composition of pequi extracts. C1X0 - Obtained in the first collector with SFE at 400 MPa and 333 K. C2X0 - Obtained in the second collector with SFE at 400 MPa and 333 K. C1CP - Obtained in the first collector with SFEAP (40Nm) for 1 min at 400 MPa and 333 K.

| Fatty acids (% m/m) | C1X0 | C2X0 | C1CP |
|---|---|---|---|
| C 8:0 (Caprylic) | 0.0 (±0.0) | 0.0 (±0.0) | 0.1 (±0.0) |
| C 10:0 (Capric) | 0.0 (±0.0) | 0.0 (±0.0) | 0.1 (±0.0) |
| C 12:0 (Lauric) | 0.1 (±0.0) | 0.2 (±0.0) | 0.3 (±0.0) |
| C 14:0 (Myristic) | 0.1 (±0.2) | 0.1 (±0.1) | 0.4 (±0.0) |
| C 16:0 (Palmitic) | 32.0 (±0.0) | 31.9 (±0.1) | 34.7 (±0.2) |
| C 16:1 (Palmitoleic) | 0.6 (±0.0) | 0.6 (±0.0) | 0.4 (±0.4) |
| C 17:0 (Margaric) | 0.1 (±0.0) | 0.1 (±0.0) | 0.1 (±0.0) |
| C 17:1 (cis-10-Heptadecanoic) | 0.1 (±0.0) | 0.1 (±0.0) | 0.1 (±0.0) |
| C 18:0 (Stearic) | 2.4 (±0.3) | 2.2 (±0.0) | 2.2 (±0.1) |
| C 18:1 (Oleic) | 61.4 (±0.5) | 61.4 (±0.3) | 58.0 (±0.8) |
| C 18:2 (Linoleic) | 2.3 (±0.0) | 2.3 (±0.1) | 2.5 (±0.3) |
| C 18:3 (Linolenic) | 0.4 (±0.0) | 0.4 (±0.0) | 0.5 (±0.1) |
| C 20:0 (Arachidic) | 0.1 (±0.1) | 0.2 (±0.0) | 0.2 (±0.0) |
| C 20:1 (Eicosenoic) | 0.3 (±0.0) | 0.3 (±0.0) | 0.2 (±0.1) |
| C 22:0 (Behemic) | 0.0 (±0.0) | 0.1 (±0.0) | 0.1 (±0.0) |
| C 24:0 (Lignoceric) | 0.1 (±0.0) | 0.1 (±0.0) | 0.1 (±0.0) |

It is interesting to mention that the fatty acid composition of extract from pequi by SFE is comparable with that of olive oil. Their palmitic acid composition are 32% for pequi extract in comparison with 12.8% for olive extract, and their Oleic composition are 61% for pequi extract in comparison with 59.3% for olive extract. However, linoleic fatty content in pequi extract with SFE is 8.4 times smaller than that of olive extract [30]. Added to this, the overall extraction yield from ground olives and pequi are 33% and 53.4%, respectively [30, 31].

After the extraction processes, the raw material was removed from the extractor for protein analysis. It was found that it contained 20% ± 0.55 of protein, and was denominated as pequi protein meal.

## 4 CONCLUSION

In this work, the new extraction method SFEAP is presented for the first time and is shown to offer a faster extraction rate than SFE with lower amounts of solvent. To technically validate the SFEAP method, the method was used for pequi, and its performance was compared with that of SFE. Prior to performing SFEAP, pequi extraction using SFE was performed at temperatures of 313 and 333 K and pressures of 20, 25, 30, 35, and 40 MPa to determine the optimum temperature and pressure. It was found that a temperature of 333 K and pressure of 40 MPa gave the highest SFE yield, 48 g extract/100 g raw material. Comparing the SFEAP yield at this optimum operating condition with the SFE yield revealed that the SFEAP yield was eight times greater than that of SFE during the first minute of extraction. This paper highlights the importance of cold pressing before performing SFE, and in particular, the SFEAP method is recommended for extraction of other raw materials with high oil contents.

## 5 ACKNOWLEDGEMENTS

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# CAPÍTULO 6

*DISCUSSÕES GERAIS*

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O equipamento montado apresentou custo inferior em relação aos principais equipamentos comerciais disponíveis no mercado. Vários trabalhos foram desenvolvidos com o equipamento inclusive operando com uma bomba HPLC adicional para um cosolvente, demostrando a flexiblidade da unidade na extração com diferentes solventes assitida ou não por prensagem e fracionamento. Uma coluna de até 1000 mL poderia ser utilizada em substituição ao extrator de 100 mL sem prejudicar o funcionamento do equipamento. A estrutura montada gerou resistência suficiente para suportar torques de 150 Nm sem deformações dos perfilados.

O uso de uma válvula de contrapressão para cada célula de pressão permitiu um controle preciso das pressões de trabalho e o sistema que aqueceu as válvulas pelo fluido (água) apresentou maior eficiência do que os sistemas que aqueciam as válvulas por resistência elétrica comumente utilizados em outros equipamentos.

A validação dos separadores utilizando o funcho resultou na obtenção de uma fração lipídica de coloração esverdeada no primeiro separador e um óleo essencial extremamente aromático e incolor no segundo separador. Este resultado positivo de fracionamento possibilitou a continuação dos estudos de fracionamento de outras matérias-primas.

O aprimoramento da técnica de Cromatografia em camada delgada permitiu relizar as análises dos extratos obtidos e comparar os compostos bioativos obtidos no primeiro e segundo separadores. A utilização do Software ImageJ como ferramenta de tratamento das imagens obtidas permitiu realizar as medidas das placas com exatidão e também avaliar a intensidade de cada banda.

Os procedimentos elaborados para a operação do equipamento possibilitaram maior confiabilidade nos resultados obtidos, que somada à experiência adquirida durante as etapas de montagem e validação resultaram no fracionamento do extrato supercrítico de urucum em extratos com composição de carotenoides diferentes, principalmente em relação à bixina. Os resultados de fracionamento do extrato de urucum e equilíbrio de fases das frações serviram de base para a continuação deste trabalho na determinação de outras condições de fracionamento e suas respectivas composições.

O estudo de extração e fracionamento do óleo de pequi resultou no desenvolvimento de um novo processo de extração, SFEAP – Supercritical Fluid Extraction assisted by Pressing. Dentre as etapas do planejamento desenvolvido duas adaptações realizadas no equipamento foram fundamentais para obtenção destes resultados. A primeira foi relacionada à coleta do extrato, onde foram adotados dois frascos em série para coleta e a segunda adaptação foi a construção de uma prensa visando obter rendimentos de extração maiores já no início do processo de extração.

As adaptações técnicas desenvolvidas para aumentar o rendimento e melhorar o controle da pressão nos frascos aplicadas para a extração supercrítica da polpa de pequi possibilitaram o monitoramento da pressão no primeiro frasco de coleta e eliminaram a perda de extrato devido ao arraste juntamente com o $CO_2$ até o rotâmetro.

A prensa construída, além de permitir extração prévia da matéria-prima dentro do leito de extração seguida da extração com solvente, pode ser aplicada para controlar a força que será aplicada sobre o leito de extração durante a etapa de empacotamento, sendo apenas necessária a utilização de um torquímetro de faixa de torque menor em relação ao utilizado para prensagem.

O processo SFEP pode ser aplicado em outras matérias-primas, inclusive aplicando fracionamento em série após a extração com solvente. A prensa também pode ser utilizada previamente ao processo de extração com diferentes solventes aplicando-se uma segunda bomba no equipamento.

A composição em ácidos graxos dos extratos de pequi obtidos foi próxima aos trabalhos encontrados na literatura científica, apresentando 61% de ômega 9 e com um rendimento de extração de 53.4% (m/m). Os extratos obtidos no primeiro e no segundo frascos de coleta do processo SFE apresentaram perfis de ácidos graxos semelhantes. O sólido retirado do extrator após a extração tem 20% de proteína e pode ser comercializado como uma farinha proteica.

# CAPÍTULO 7

*CONCLUSÕES GERAIS*

# CAPÍTULO 7

## *CONCLUSÕES GERAIS*

A etapa de montagem da unidade SFE-0.1L começou com a pesquisa das empresas nacionais que comercializavam as peças necessárias para o desenvolvimento do trabalho. Alguns fornecedores já tinham realizado vendas para o LASEFI, porém o levantamento não esteve baseado somente nas empresas conhecidas e buscou novos fornecedores com o menor custo possível e a maior aplicabilidade.

Somente referente à montagem da estrutura de perfilados do equipamento partindo de um fornecedor foram encontrados mais quatro empresas que trabalham com este tipo de material. Desta forma foi possível constatar que cada empresa trabalhava com matérias de dimensões, qualidades e preços diferentes. A utilização de perfilados de menor espessura (30 × 30 mm) foi fundamental no desenvolvimento do projeto da unidade visto que perfilados menores apresentam menor custo e formam uma estrutura com um maior espaço interno para a acomodação das peças.

A etapa de validação da Unidade SFE-0.1L foi importante para demonstrar a operacionalidade do equipamento desenvolvido. O equipamento construído pode ser utilizado para extrair diferentes matérias-primas, incluindo estudos com e sem os separadores, aplicando uma coluna de extração com volume de até 1 L e aplicando uma bomba HPLC para extração com outros solventes.

A montagem de um equipamento de extração supercrítica foi financeiramente mais vantajosa em relação à aquisição de uma unidade comercial e apresentou uma operacionalidade satisfatória permitindo a extração e separação dos compostos estudados.

A etapa de validação de um método de quantificação dos extratos de urucum possibilitou a análise dos extratos de forma rápida e eficiente. A produção de um padrão de bixina possibilitou uma boa estimativa das concentrações deste carotenoide em cada um dos extratos coletados durante as cinéticas de extração e fracionamento do extrato bruto em dois produtos diferentes.

O método validado de utilização do software pode ser modificado e utilizado para extratos de outras matérias-primas e o procedimento desenvolvido pode ser utilizado para produção se um sinal analítico em pixels em outras técnicas que envolvam fotodocumentação como, por exemplo, a eletroforese SDS-PAGE e PCR - Polymerase Chain Reaction. O método de obtenção de medidas pelas imagens no programa ImageJ também pode ser

utilizado em outras técnicas que envolvam o cálculo do $R_f$ ou na obtenção de qualquer medida analítica em que se tenha uma escala referencial na imagem utilizada.

Por fim o equipamento pode ser adaptado para a acoplagem de uma prensa em seu extrator. Os estudos realizados com esta prensa possibilitaram a avaliação do efeito da prensagem no processo de extração e a determinação da força que deve ser aplicada ao leito para não prejudicar a etapa posterior de extração com solventes.

A extração com fluido supercrítico assistida por prensa da polpa de pequi é uma novo processo que permite a obtenção do extrato com menores demandas de solvente e em menor tempo. O óleo obtido pode ser aplicado na indústria de cosméticos, alimentícia, farmacêutica e o subproduto gerado da extração pode ser comercializado como uma farinha proteica de pequi. O processo desenvolvido é indicado para aplicação a outras matérias-primas que possuam elevados teores de óleo em sua composição.

# MEMÓRIA DO PERÍODO DE DOUTORADO

## MEMÓRIA DO PERÍODO DE DOUTORADO

O doutorando Júlio Cezar Johner Flores ingressou na Unicamp em 2013 através do processo seletivo do Departamento de Engenharia de Alimentos (DEA). Com Auxílio financeiro do CNPq (Processo nº 140287/2013-2), cursou as 4 disciplinas obrigatórios: TP-320 Termodinâmica; TP-322 Fenômenos de Transporte I; TP-323 Fenômeno de Transporte II; TP-199 Seminários e três disciplinas optativas oferecidas no respectivo departamento: TP-132 Métodos Matemáticos na Engenharia de Alimentos; TP-276 Tratamento Biológico de Águas Residuárias; TP-159 Tópicos Especiais em Engenharia de Alimentos (Ciclo de Aprendizagem PDSA), totalizando 21 créditos.

O doutorando participou em 2013 do curso de instalação e operação do espectrofotômetro ultravioleta e visível modelo 800XI. Em 2015 o doutorando participou do curso, ministrado pelo técnico da Jasco, de treinamento para operação do equipamento SFC - Supercritical Fluid Chromatography (Jasco, Detector ELS 2040 Evaporative Light Scattering Detector - CO-2065 plus intelligent Column oven - BP 2080 plus automatic back pressure regulator, São Paulo, Brasil).

O doutorando participou do treinamento de operação do equipamento SFC - Supercritical Fluid Chromatography (Jasco, Detector ELS 2040 Evaporative Light Scattering Detector - CO-2065 plus intelligent Column oven - BP 2080 plus automatic back pressure regulator, São Paulo, Brasil) em que foi desenvolvido um método para determinação de bixina pelo padrão utilizado nas análises de TLC

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## DEPÓSITO DE PATENTE

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# *APÊNDICES*

# Procedimento Operacional Padrão (POP)

# Fluxômetro

Autor: Júlio Cezar Johner Flôres

Campinas-SP

## Procedimento Operacional Padrão do Fluxômetro

O fluxômetro de corpo em suspenção, tipo rotâmetro, é um equipamento robusto que só necessita de reparo devido ao uso inadequado. Este tipo de equipamento deve ser utilizado somente para aferição de gases sem contaminações com extratos que venham a prejudicar seu funcionamento. Para seu bom funcionamento nenhuma partícula de extrato pode estar presente entre a esfera e o vidro prejudicando a medida, para evitar danos ao fluxômetro são recomendados mais testes na etapa de separação do extrato. Para abertura do fluxômetro é necessária somente uma chave philips que possibilite a abertura dos 12 parafusos que fixam a entrada e saída do medidor.

Procedimento operacional padrão fluxômetro:

1. Proceda com a remoção do fluxômetro da unidade desconectando as mangueiras de entrada e saída do equipamento e posteriormente as duas porcas conforme ilustrado na figura A1;
2. Remova a tampa frontal com uma chave philips (Figura A2.A). As tampas laterais dão suporte ao tubo que aloja a esfera metálica, antes de remover os quatro parafusos de cada tampa lateral coloque a carcaça do fluxômetro em um local forrado com papel para evitar que a queda da corpo ou do tubo venha a danificar estes frágeis componentes. Remova os parafusos restantes (figura A2.B) e separe as peças (Figura A3.A).
3. Abra a entrada (figura A3.B) e faça a remoção do filtro para limpeza (figura A3.C).
4. Limpe todos os componentes com água e detergente e por fim com álcool.
5. Faça a montagem da entrada do fluxômetro seguindo a sequência de peças da figura A3.B.
6. Centralize o tubo de vidro nas peças de entrada e saída do fluxômetro de forma que as duas pontas do tubo estejam completamente tampadas pelas vedações de borracha.
7. Parafuse as duas laterais.
8. Parafuse a tampa frontal
9. Parafuse a tampa traseira.
10. Instale no equipamento e reconecte as mangueiras de entrada e saída.

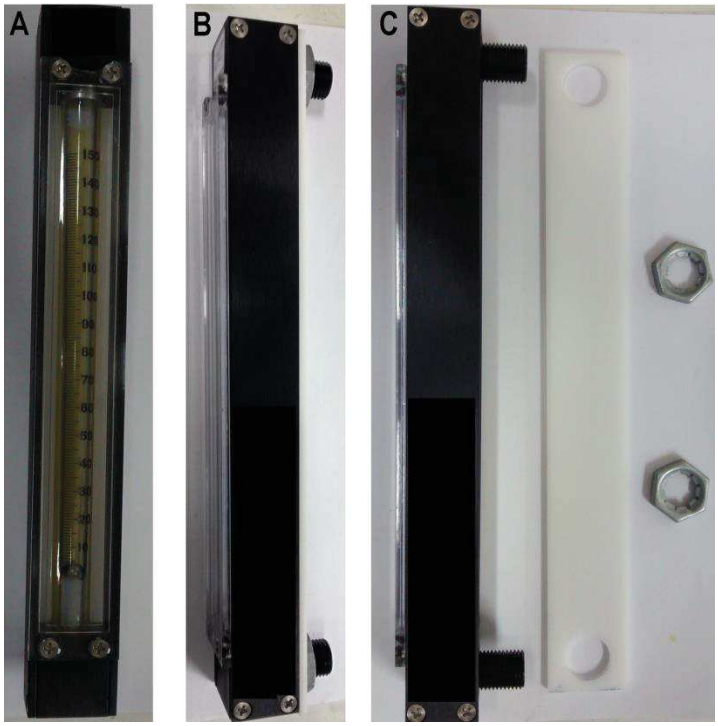


**Figura A1 -** Fluxômetro. (A) Vista frontal, (B) Vista lateral e (C) Tampa traseira e porcas.

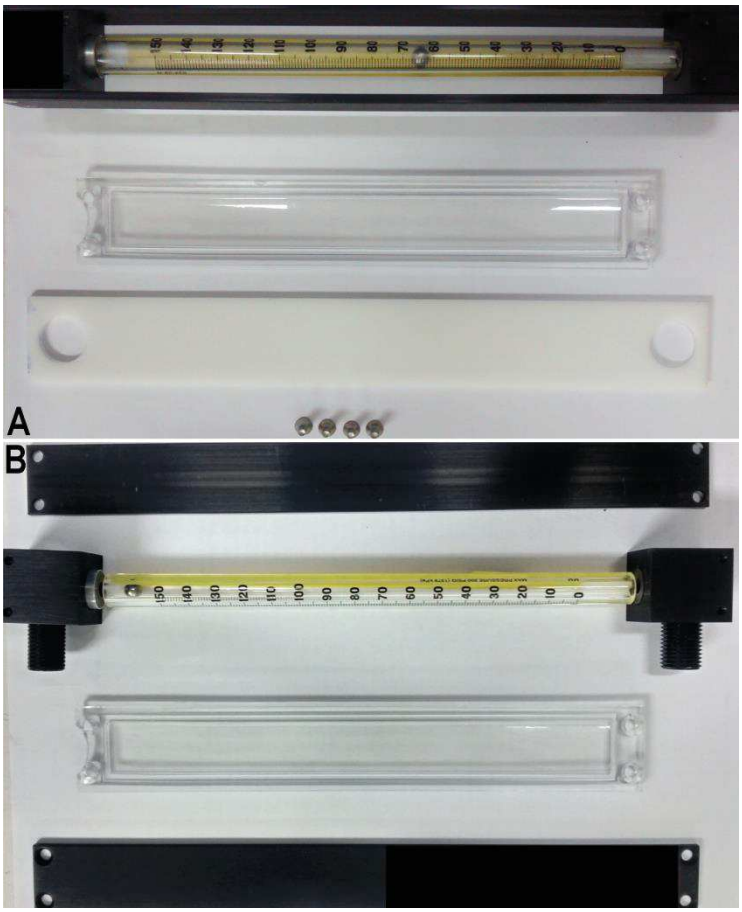


**Figura A2 -** Fluxômetro desmontado. (A) Remoção da tampa frontal e (B) Remoção das placas laterais

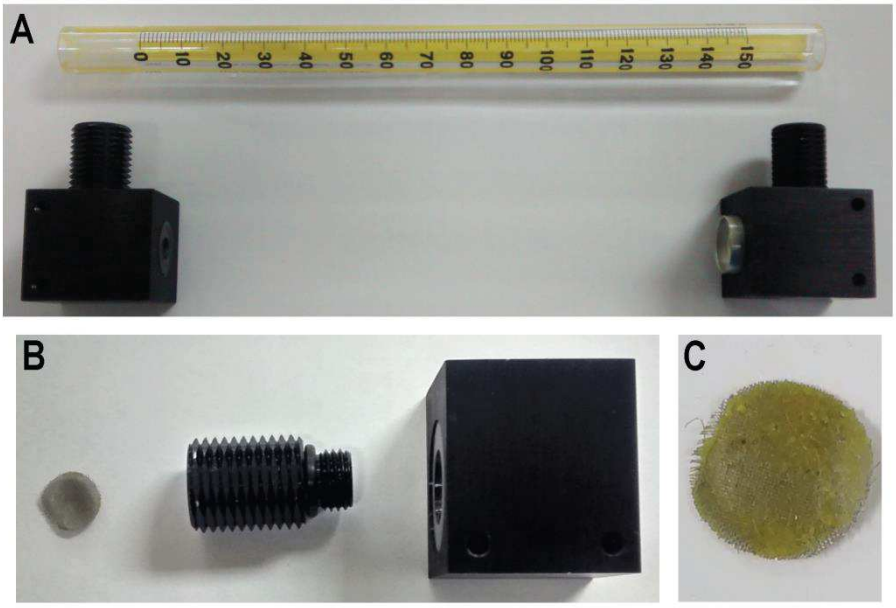


**Figura A3** - Componentes do fluxômetro. (A) Entrada, tubo e saída (Na ordem direita para esquerda); (B) Entrada desmontada (C) Filtro metálico

# Procedimento Operacional Padrão (POP)

# Válvula Back Pressure

Autor: Júlio Cezar Johner Flôres

Campinas-SP

## Procedimento Operacional Padrão Válvula Back Pressure

O procedimento de limpeza e manutenção de uma válvula Back Pressure deve ser realizado sempre que este componente não responda de forma progressiva ao movimento de sua alavanca em uma resposta de aumento na pressão, caracterizando um problema de entupimento ou que a vedação está travada na agulha. Nunca deve ser exercido torque elevado na alavanca superior da válvula, esta válvula possui um sistema com mola que permite com baixo torque manter pressões de 400 bar. Toda manutenção feita na válvula deve ser realizada com a válvula completamente aberta para que durante a etapa de montagem as peças não sejam danificadas. A válvula Back Pressure é responsável pela manutenção da pressão dentro do extrator liberando para o reciclo o $CO_2$ necessário para manter a pressão de extração determinada. Caso esta válvula não esteja mantendo a pressão no extrator o $CO_2$ bombeado será reciclado e a pressão na linha após a bomba será sempre a mesma que a da alimentação de $CO_2$.

Procedimento operacional padrão válvula Back Pressure:

1. Proceda com a remoção do painel de válvulas da unidade para ter acesso ao corpo de válvula;
2. Retire a alavanca superior da válvula, desconecte as tubulações de entrada e saída conforme as etapas ilustradas na figura A4 e A5. Retire as braçadeiras que matêm a válvula fixa.
3. Com a válvula solta e fixada na morsa utilize uma chave de boca para abrir o corpo da válvula, conforme ilustrado na figura A6.
4. Remova a mola e o pistão da válvula, conforme ilustrado na figura A6 e A7.
5. Faça a remoção do centro da válvula com um chave de fenda, nesta etapa utilize uma chave de fenda de tamanho apropriado, conforme figura A8 (o maior tamanho que entre na fenda para evitar danos à cabeça do parafuso e possibilitar maior torque).
6. Limpe todos os componentes da válvula utilizando água e detergente seguido de etanol para remover completamente qualquer fuligem metálica das roscas.
7. Reconecte as partes conforme ilustrado nas figuras A10 e A11.

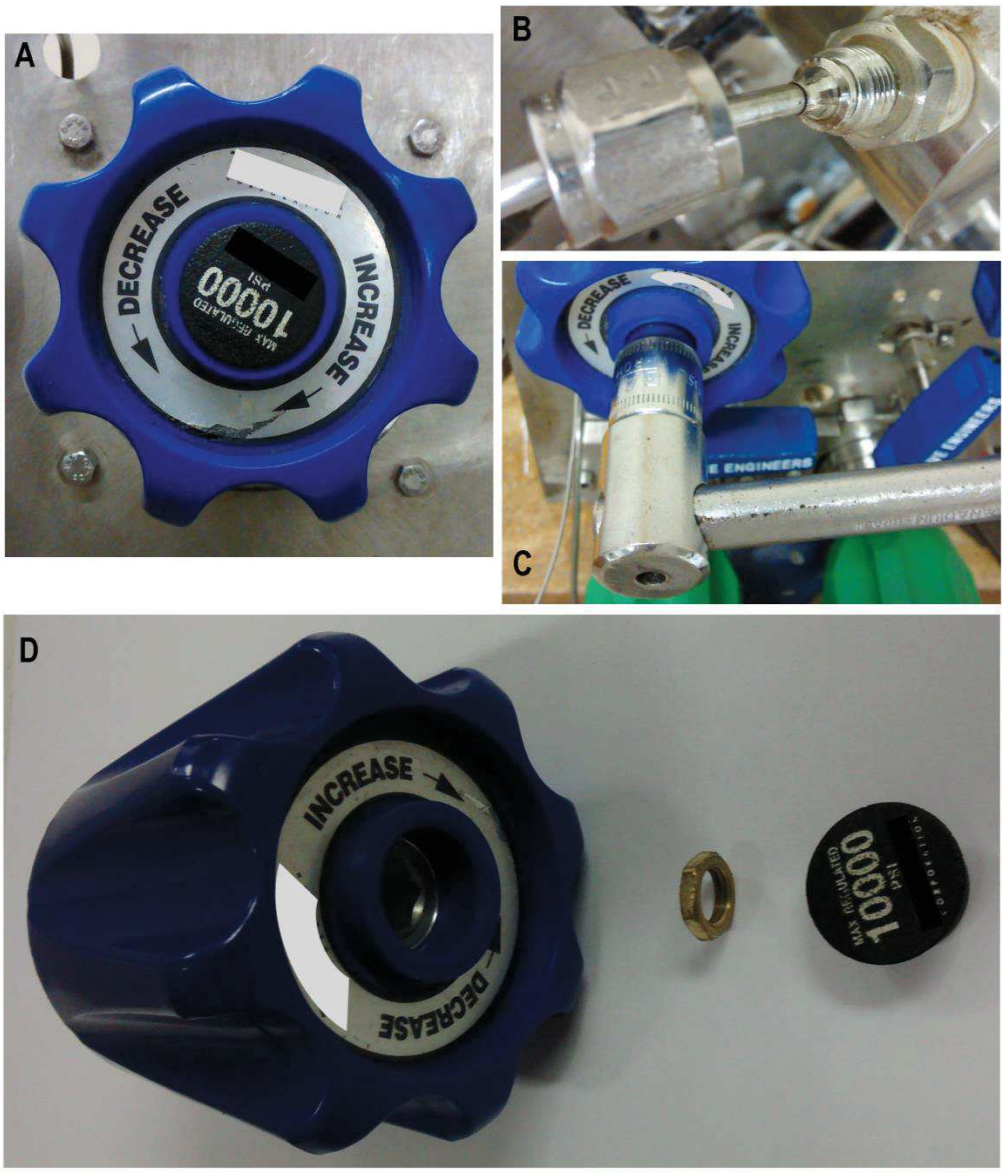


**Figura A4** - Remoção dos componentes periféricos da válvula. A - Válvula montada, B - Abertura das conexões de entrada e saída, C - Desmontagem da alavanca de válvula e D - Peças na ordem de montagem.

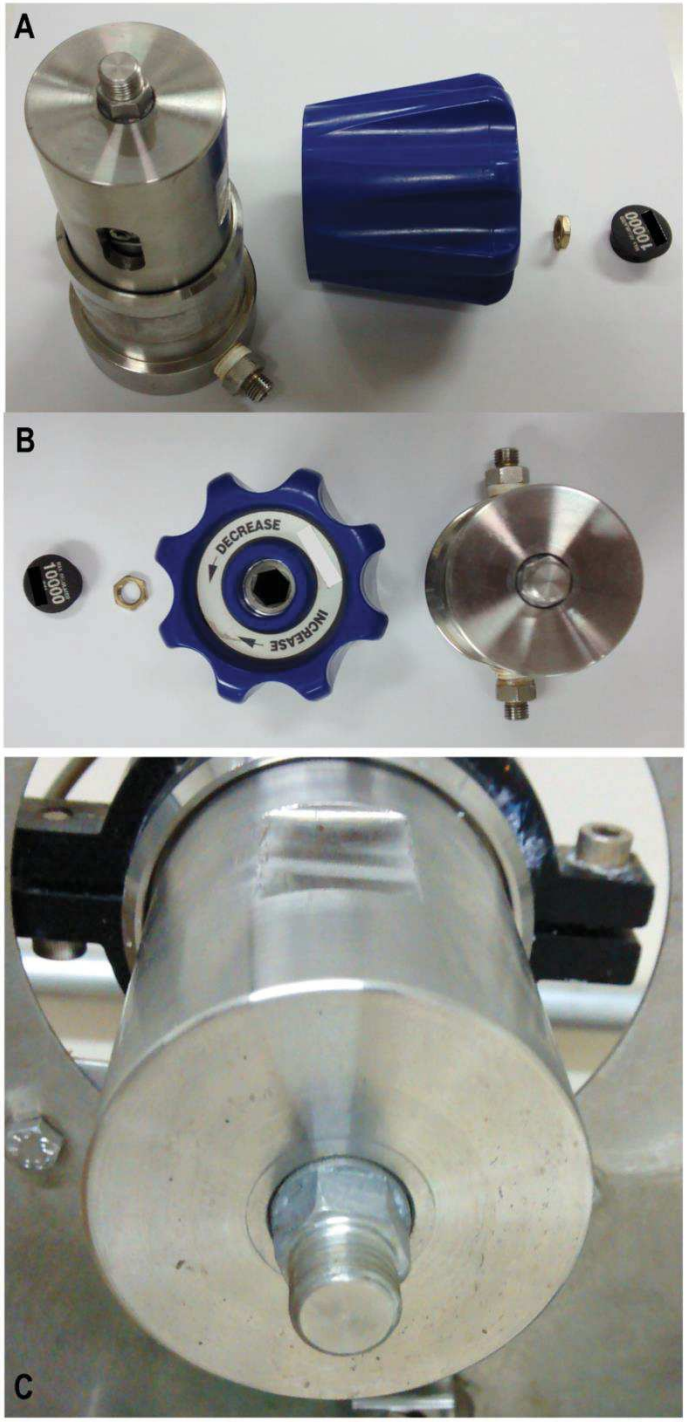


**Figura A5** - Remoção da válvula do equipamento. A - Vista lateral das peças, B - Vista superior das peças e C - Abertura do suporte da válvula.


**Figura A6** - Abertura do corpo da válvula. A - Fixação da válvula na morsa, B - Posição da chave inglesa para abertura e C - Peças obtidas.

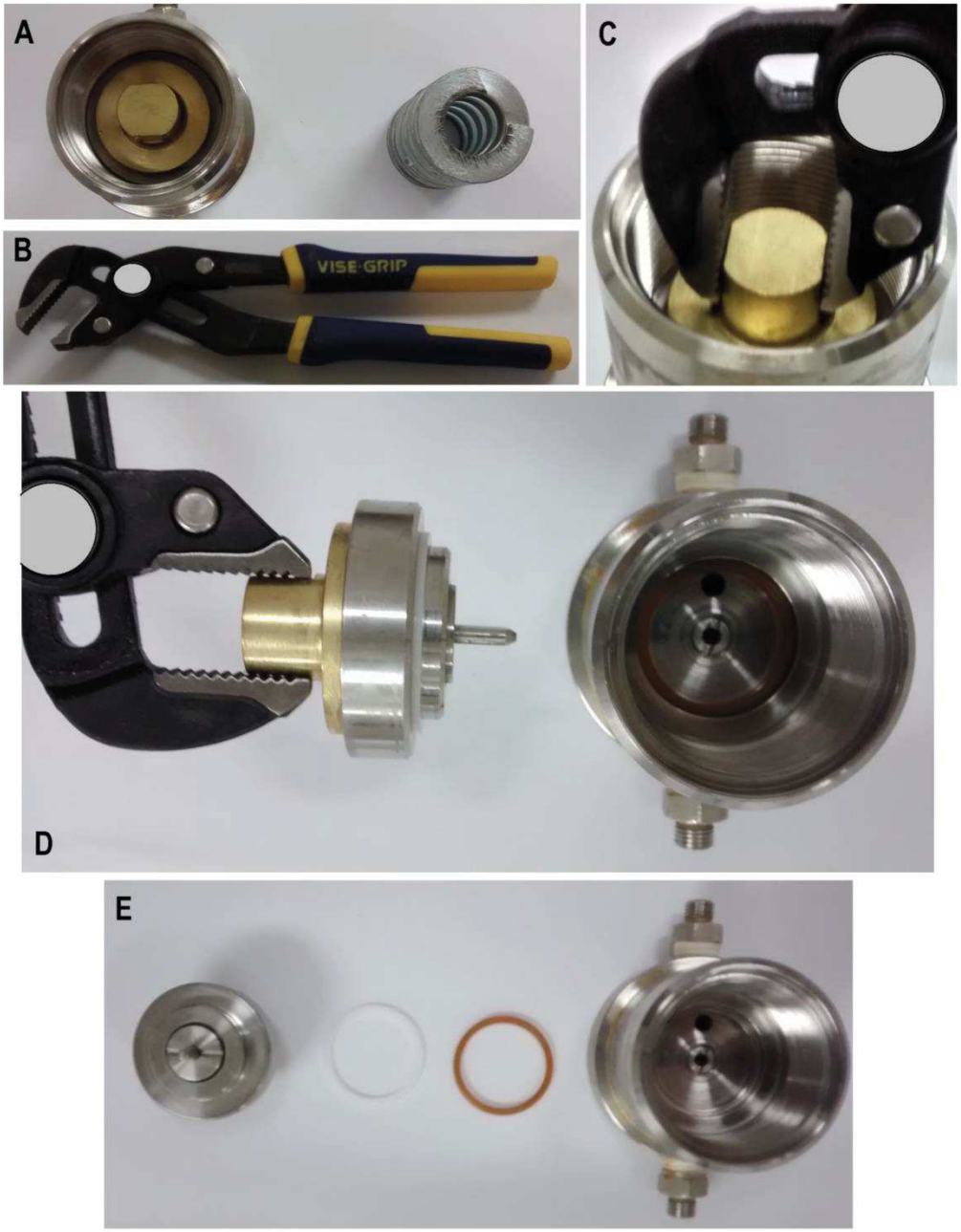


**Figura A7** - Remoção da mola e pistão da válvula. A - Remoção da mola, B - Ferramenta para a etapa C, C - Fixação da ferramenta, D - Remoção do pistão e E - Vista superior das peças.


**Figura A8** - Remoção do tubo de conexão da válvula. A - Tubo montado, B - Abertura do tubo, C – Vedação de poliacetal (Arruela) e D - Tubo de conexão.

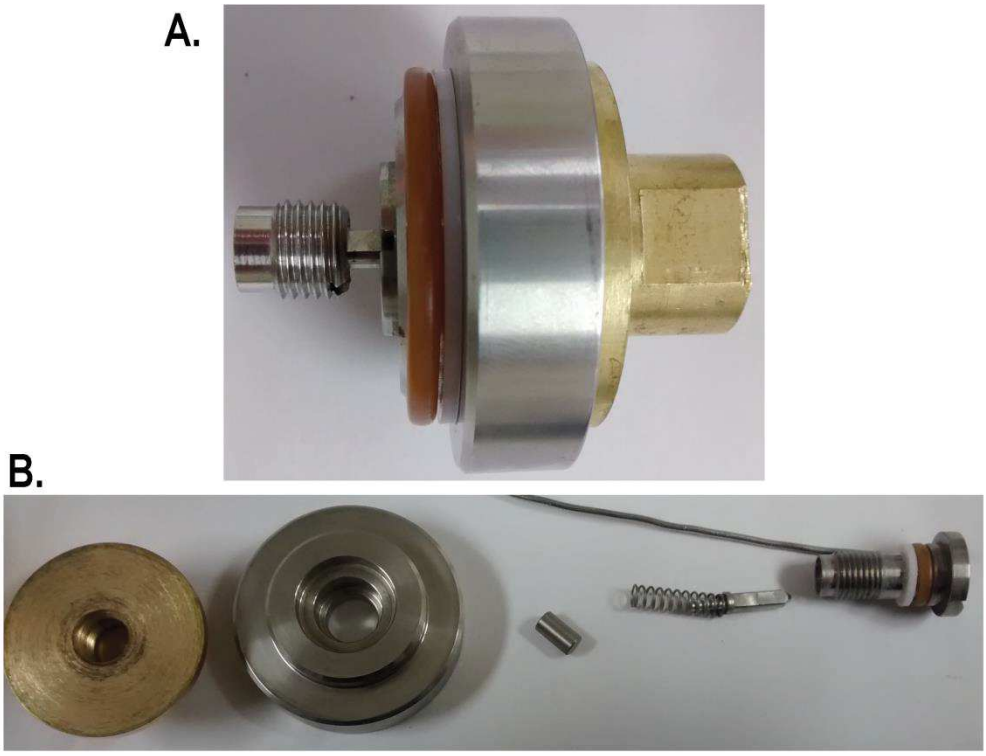


**Figura A9** – Êmbolo da válvula Back Pressure. A - Posição que a haste flexível deve ficar com a válvula montada. B – Sequência de montagem das peças do êmbolo da válvula.


**Figura A10** - Montagem da válvula. A - Acoplamento do corpo da válvula, B - Posição da haste de válvula, C - Acoplamento da mola, D - Acoplamento do pistão e E - Posição do pistão no corpo da válvula.

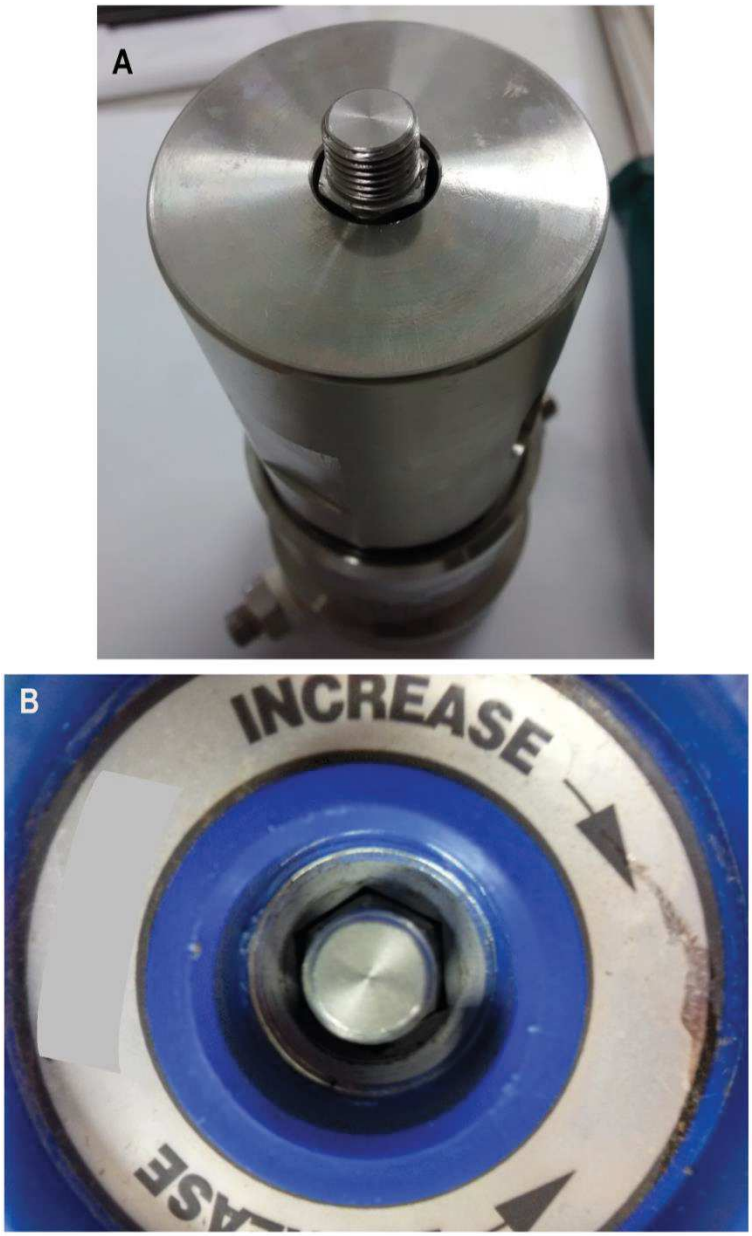


**Figura A11** - Válvula montada. A - Corpo da válvula e B - Alavanca da válvula.

# Metodologia - SFC do urucum

Durante treinamento de operação do equipamento SFC - Supercritical Fluid Chromatography (Jasco, Detector ELS 2040 Evaporative Light Scattering Detector - CO-2065 plus intelligent Column oven - BP 2080 plus automatic back pressure regulator, São Paulo, Brasil) foi padronizado um método para determinação de bixina pelo padrão utilizado nas análises de TLC. Detalhes do método podem ser observados na figura A12, em que o solvente A 70% é o metanol e o solvente B 30% $CO_2$ supercrítico.

| | |
|---|---|
| Control Method | |
| Name | Treino J |
| User Name | Administrator |
| Date Modified | 13/4/2015 14:41:20 |
| Description | |
| Method Time | 4,2 [min] |
| **Pump #1** | |
| **Initial Condition** | |
| Pump Mode | HPG2 + Iso1 |
| Flow | 3,000 [mL/min] |
| Max. Pressure | 35,0 [MPa] |
| Min. Pressure | 0,0 [MPa] |
| Solvents | |
| A | 70,0 [%] |
| B | 30,0 [%] |
| C | 0,0 [%] |
| D | 0,0 [%] |
| **Pump #3** | |
| **Initial Condition** | |
| Flow | 1,000 [mL/min] |
| Max. Pressure | 25,0 [MPa] |
| Min. Pressure | 0,0 [MPa] |
| **Pump #1 Valve / Event** | |
| **Initial Condition** | |
| Valve #1 | 1 |
| Event #1 | Off |
| Event #2 | Off |
| Event #3 | Off |
| **Column Oven** | |
| **Initial Condition** | |
| Temperature | 35,0 [deg C] |
| **ELS Detector** | |
| **Initial Condition** | |
| LED | 50 [%] |
| Gain | 1,0 |
| Evaporator Temp. | 60 [deg C] |
| Nebuliser Temp. | 40 [deg C] |
| Gas Flow | 1,60 [slm] |
| Response | 30 |
| Autozero | On |
| **Valve / Event** | |
| **Initial Condition** | |
| Valve #1 | 1 |
| Valve #2 | 1 |
| Valve #3 | 1 |
| Event #1 | Off |
| Event #2 | Off |
| Event #3 | Off |
| Event #4 | Off |
| **BPR #1** | |
| **Initial Condition** | |
| Pressure | 20,0 [MPa] |
| Temperature | 60 [deg C] |
| Event #1 | Off |
| Event #2 | Off |
| Event #3 | Off |
| Event #4 | Off |
| Control mode | SFC |

**Figura A12** - Controle do método de determinação e quantificação de bixina (Cromatografia em fluido supercrítico – SFC).

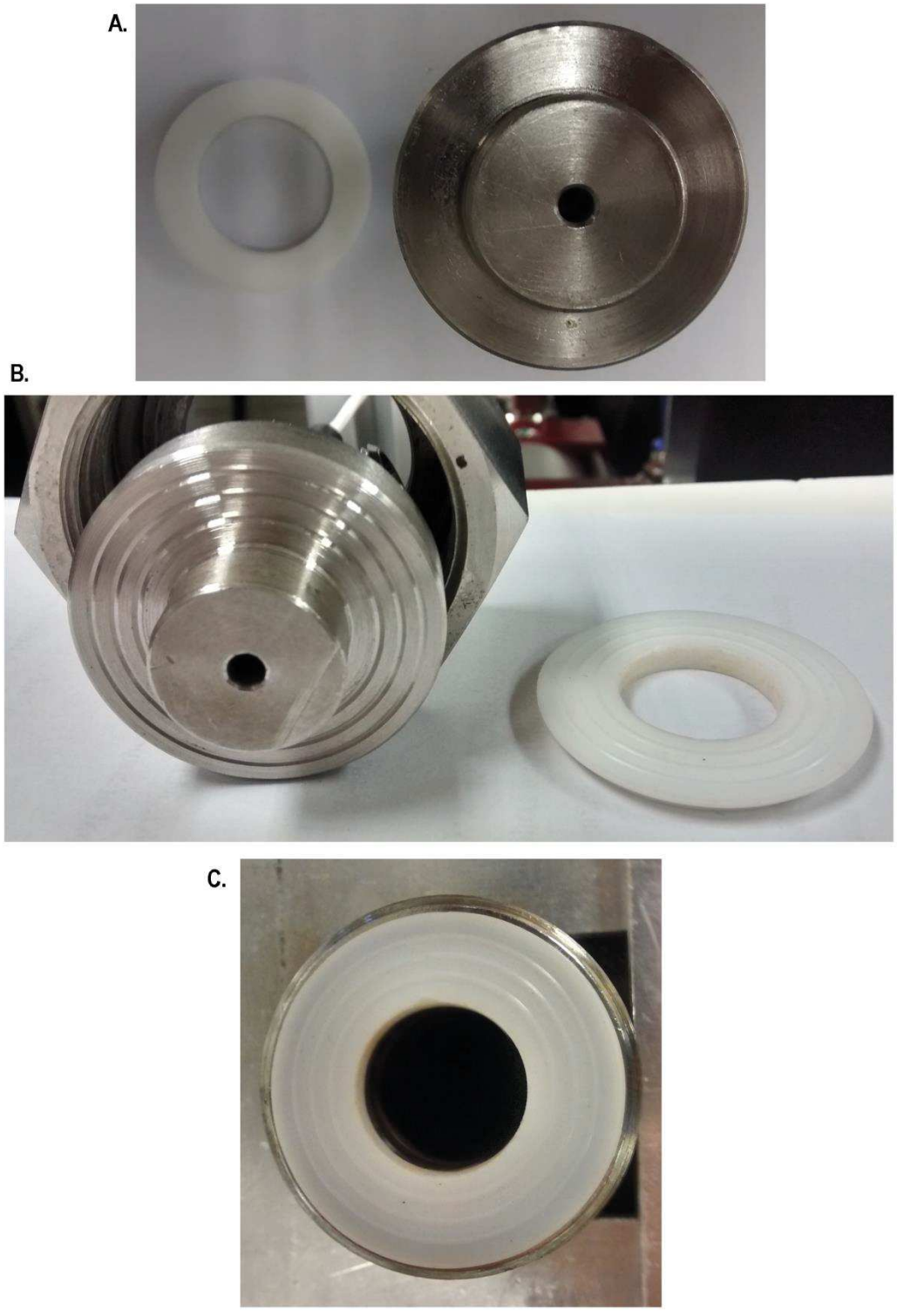


**Figura A13** - Tampa da unidade SFE-0.1L. A - Tampa da Unidade TUHH antes do reparo; B - Tampa da Unidade SFE-0.1L após aplicação das ranhuras; C - Guarnição de teflon superior deformada pelas ranhuras da tampa.

A.

B.

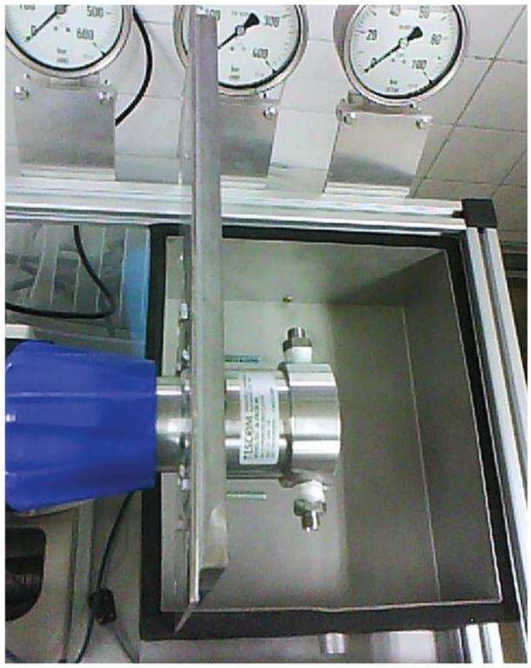

**Figura A14** - Componentes da Unidade SFE-0.1L. A. Serpentina de 6 m construída para o banho de refrigeração; B. Fixação da válvula back pressure na caixa de válvulas aquecida.

**Figura A15** - Extrato de urucum obtido nos testes iniciais de extração da unidade SFE-0.1L.

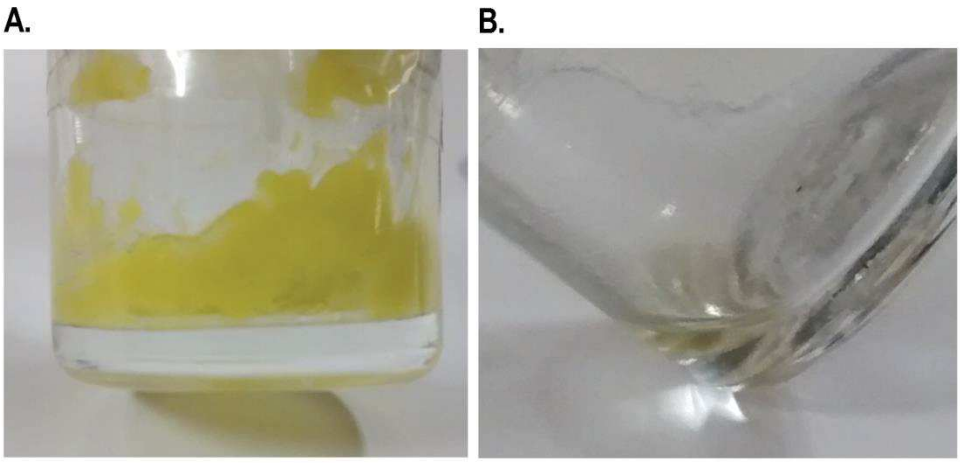


**Figura A16** - Extratos do funcho. A - Extrato obtido do primeiro separador e B - Extrato obtido do segundo separador.

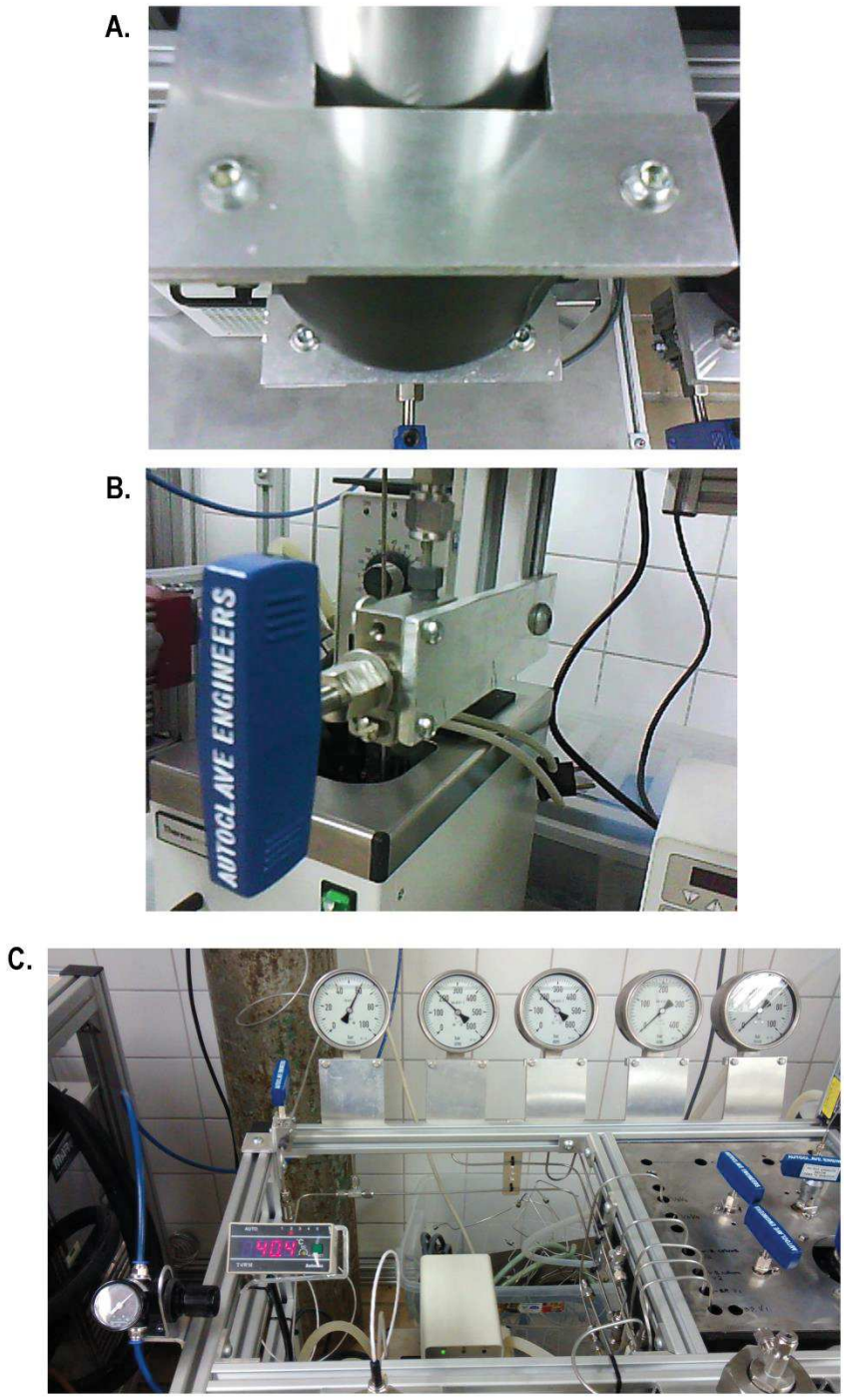


**Figura A17** - Suportes dos componentes da unidade SFE-0.1L (A) Suporte dos separadores, (B) Suporte da válvula de bloqueio e (C) Suporte do indicador de temperatura

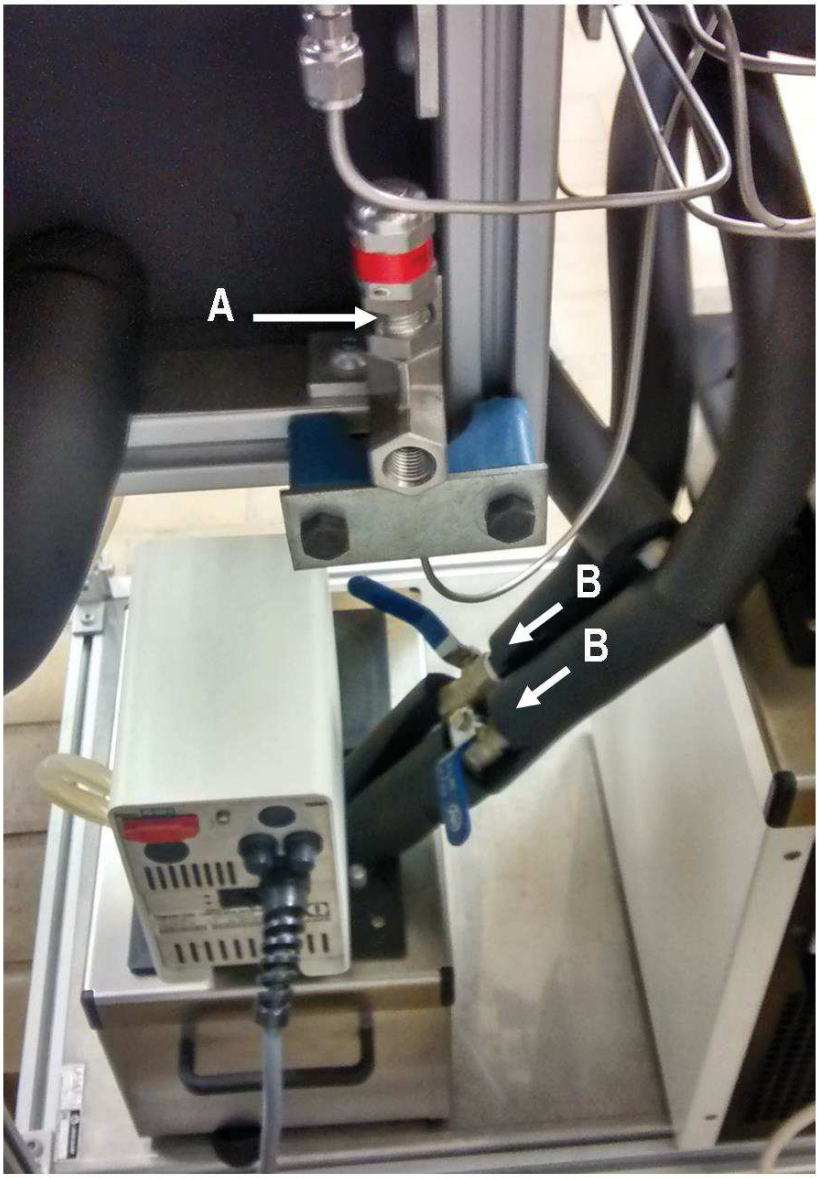


**Figura A18 -** Vista traseira da unidade SFE-0.1L. (A) Válvula de segurança, (B) Válvulas globo de entrada e saída da bomba do banho de aquecimento.

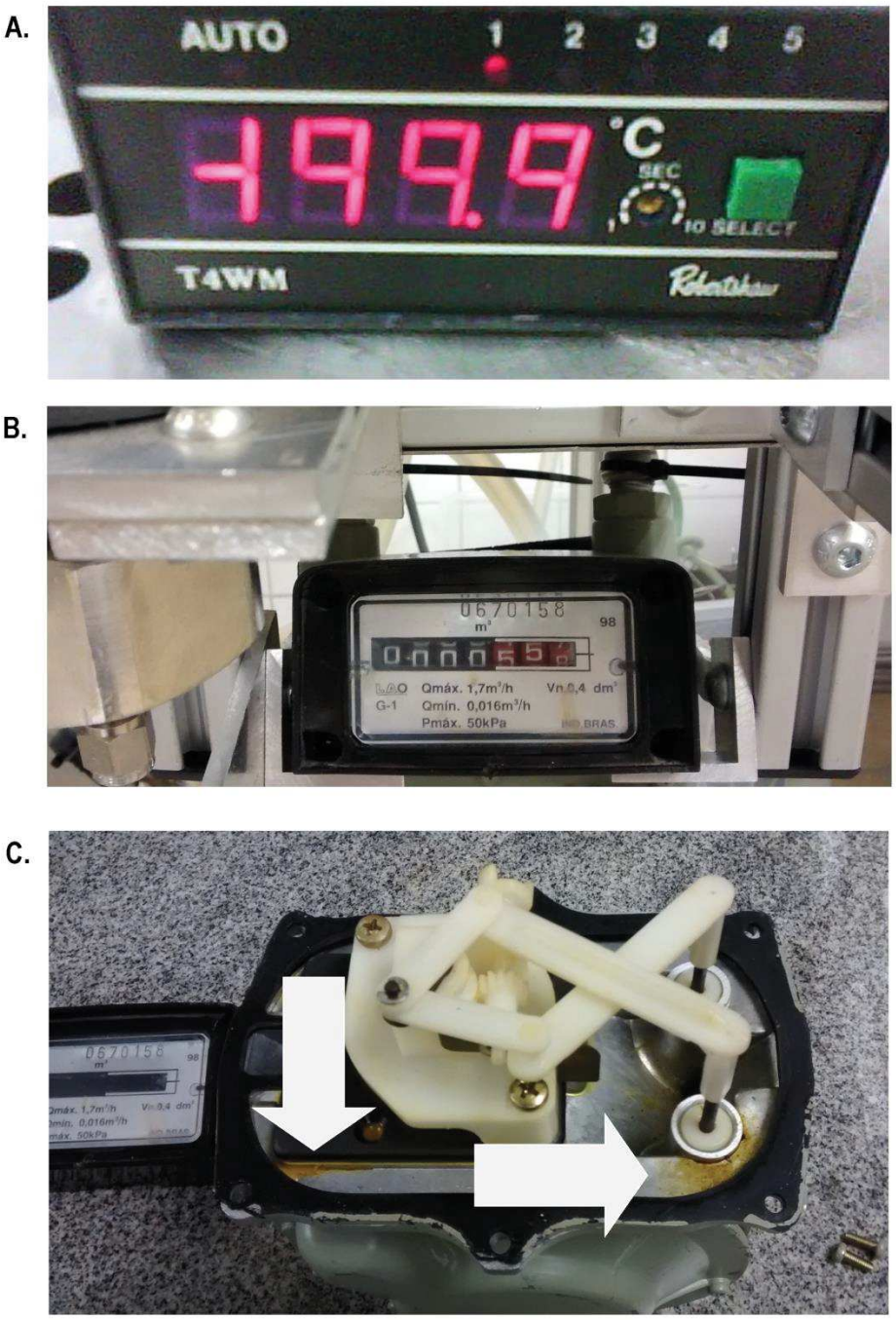


**Figura A19** - Defeitos apresentados em alguns indicadores do processo que foram solucionados com substituição por novos componentes. (A) Indicador de temperatura que apresentou defeito no visor impossibilitando a aferição da temperatura (B) e (C) Totalímetro que apresentava funcionamento irregular devido ao entupimento com extrato após anos de uso.

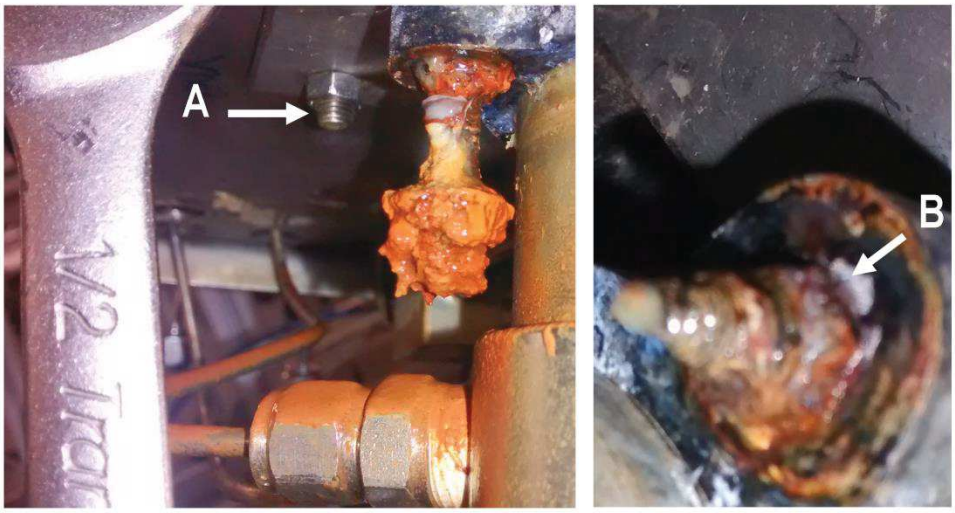


**Figura A20** – Corrosão dos parafusos de fixação das válvulas, ambos submersos por dois anos em água. (A) Parafuso de aço (B) Parafuso galvanizado.

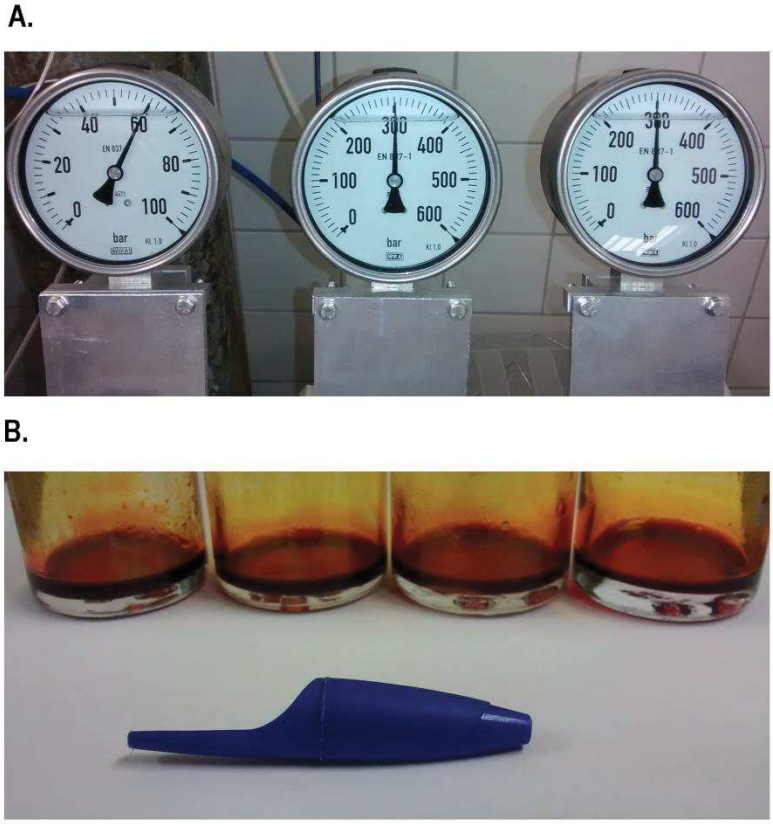


**Figura A21** - Primeiros testes desenvolvidos na unidade SFE-0.1L. (A) Teste de pressão do extrator e (B) Óleo obtido nas quatro primeiras extrações para avaliação da manutenção da temperatura de extração.

**Figura A22** – Unidade TUHH (Alemanha) antes de ser reconfigurada para SFE-0.1L.

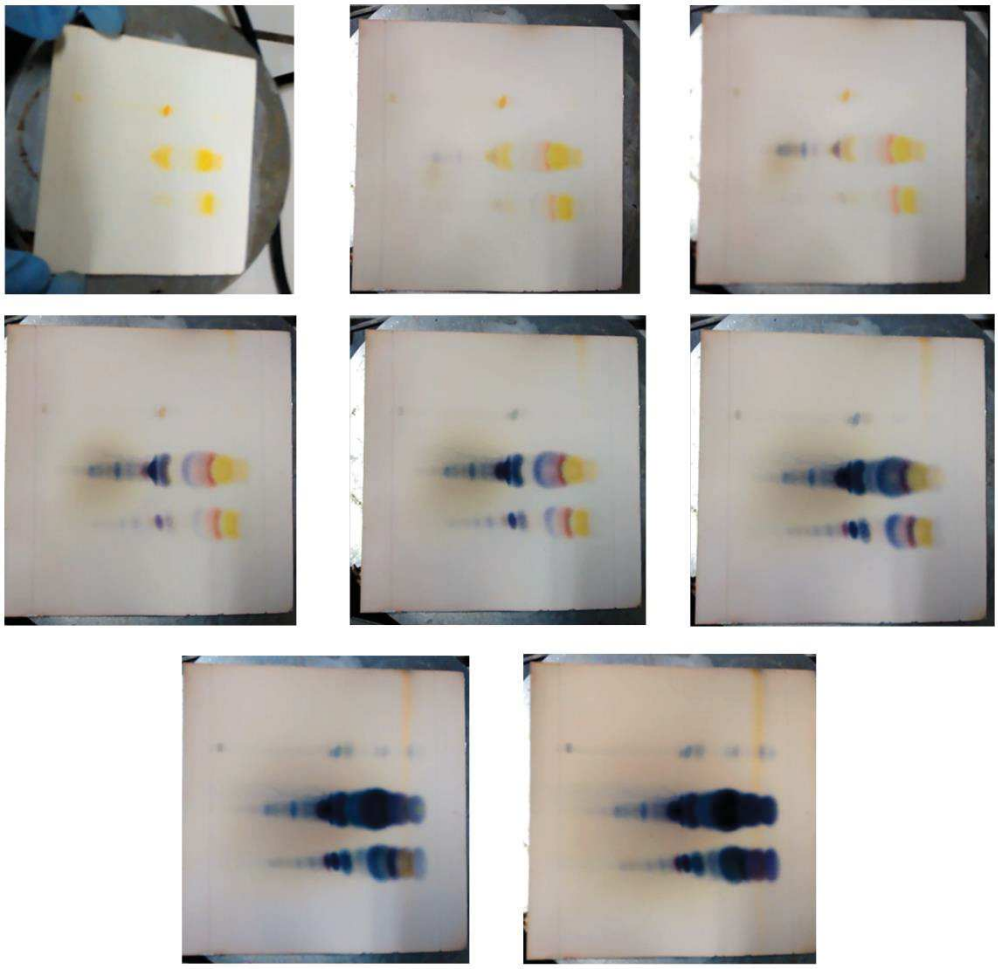

**Figura A23** – Sequência de imagens extraídas da filmagem realizada durante o aquecimento da placa de CCD após a aplicação do revelador (*p-Anisaldehyde – sulfuric* acid) iniciando na coloração amarelo-alaranjado e terminando no azul escuro.

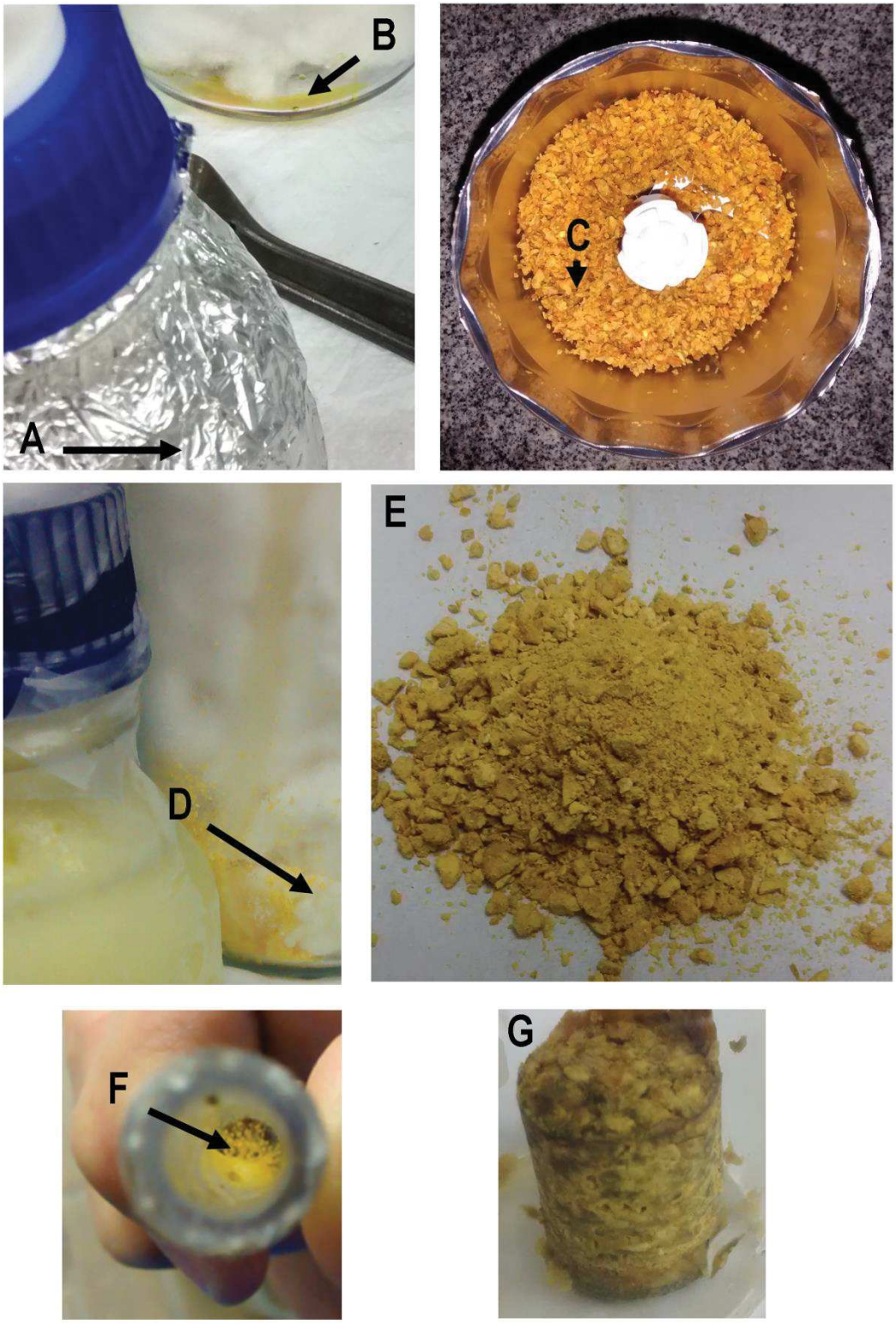


**Figura A24** – Primeiros testes de extração da polpa de pequi utilizando apenas um frasco de coleta. (A) Frasco de coleta, (B) Óleo retido no filtro de teste de extração posicionado entre o frasco de coleta (Figura A24.A) e o rotâmetro, (C) Polpa de pequi seca e triturada. (D) Extrato congelado retido no filtro de teste (E) Sólido após a extração (Farinha proteica de pequi). (F) Mangueira da saída do totalizador. (G) Matéria-prima prensada.

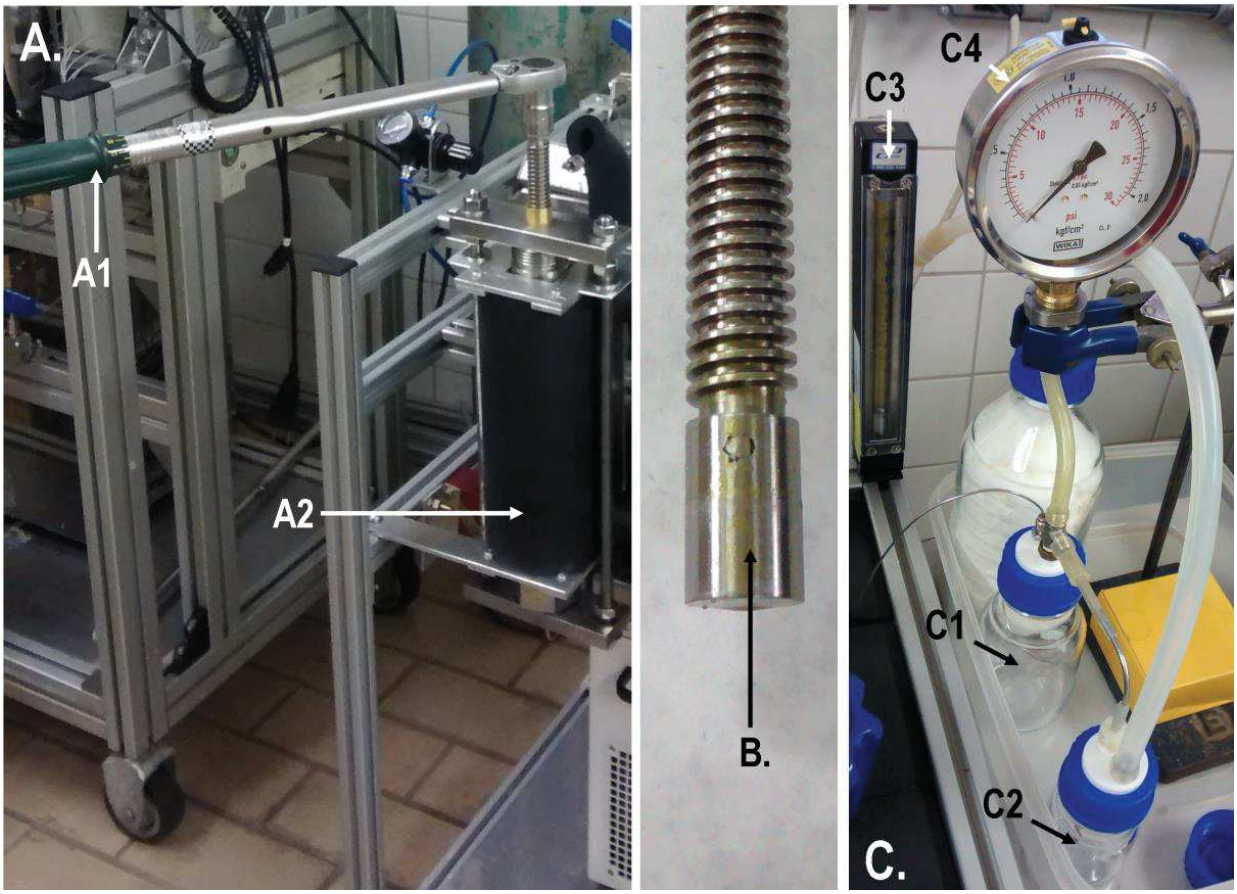


**Figura A25** - Adaptações técnicas realizadas no equipamento SFE-0.1L. A- Prensa montada, A1- Torquímetro e A2- Extrator. B. Pistão. C. Coleta dos extratos, C1- Separador 1, C2- Separador 2, C3- Rotâmetro, C4- Manômetro.

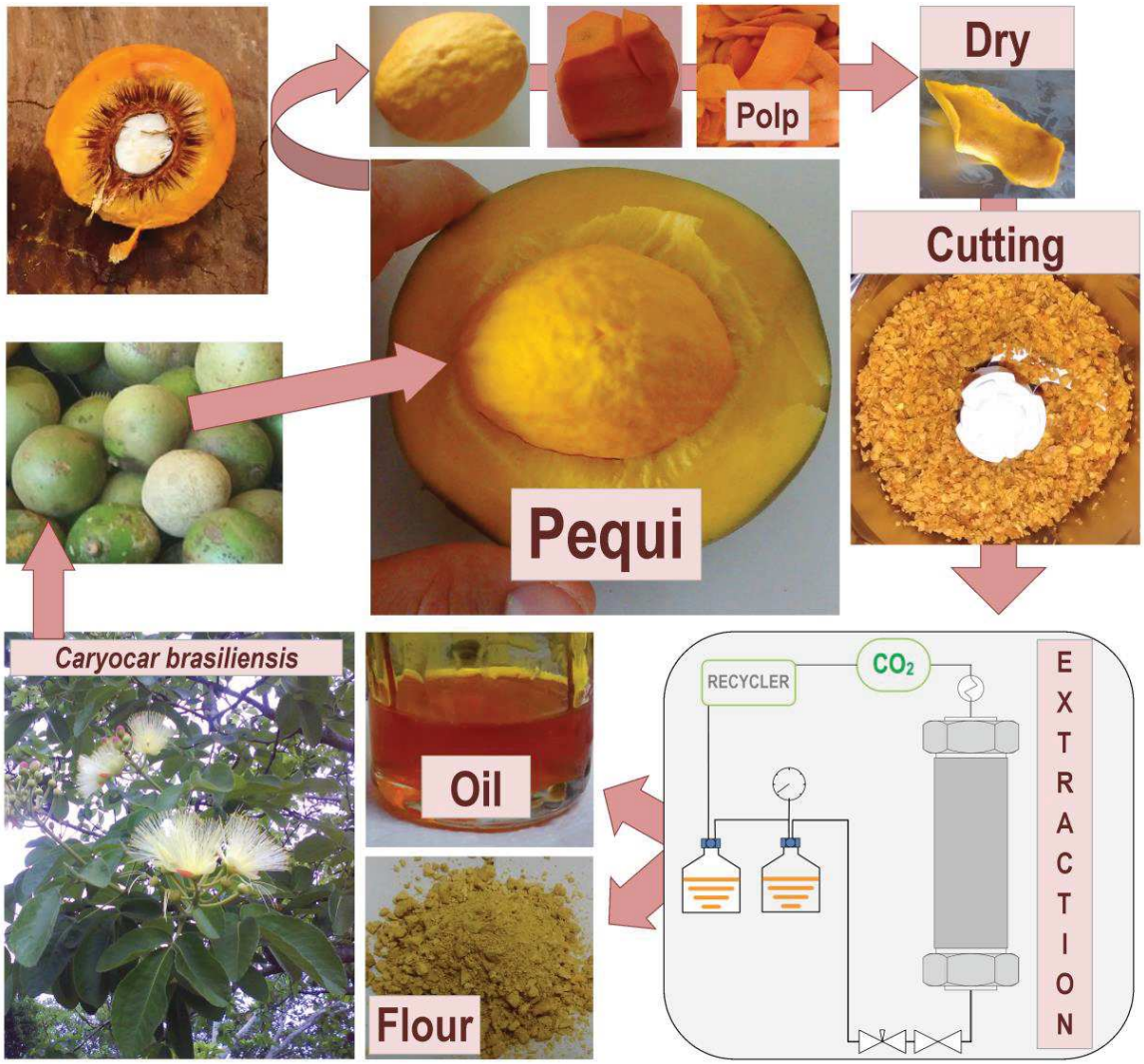


**Figura A26** - Graphical Abstract desenvolvido.

**Figura A27** – Primeiro teste de extração da polpa de pequi. O extrator foi preenchido por completo com matéria-prima e ao fim do processo o extrator foi aberto e esta foto foi registrada, ao lado o extrato obtido.

**Figura A28** – Válvula Back Pressure de haste com ponta plana. Sequência das peças que compõem uma válvula Back Pressure com vedação de encaixe aço em aço.

**Ministério do Meio Ambiente**
**CONSELHO DE GESTÃO DO PATRIMÔNIO GENÉTICO**

**SISTEMA NACIONAL DE GESTÃO DO PATRIMÔNIO GENÉTICO E DO CONHECIMENTO TRADICIONAL ASSOCIADO**

## Comprovante de Cadastro de Acesso

## Cadastro nº A9F8AFD

A atividade de acesso ao Patrimônio Genético, nos termos abaixo resumida, foi cadastrada no SisGen, em atendimento ao previsto na Lei nº 13.123/2015 e seus regulamentos.

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| Número do cadastro: | **A9F8AFD** |
| Usuário: | **Júlio Cezar Johner Flores** |
| CPF/CNPJ: | CONFIDENCIAL |
| Objeto do Acesso: | **Patrimônio Genético** |
| Finalidade do Acesso: | |

☑ **Pesquisa Científica** ☐ **Bioprospecção** ☐ **Desenvolvimento Tecnológico**

**Espécie**

**Bixa orellana**

| | |
|---|---|
| Título da Atividade: | **PROCESSO DE FRACIONAMENTO DE EXTRATO OBTIDO POR MEIO DE EXTRAÇÃO SUPERCRÍTICO DE SEMENTES DE URUCUM E USOS** |

**Equipe**

| | |
|---|---|
| **Júlio Cezar Johner Flores** | **UNICAMP** |
| **Maria Angela de Almeida Meireles Petenate** | **Unicamp** |

| | |
|---|---|
| Data do Cadastro: | **10/11/2017 12:51:07** |
| Situação do Cadastro: | **Concluído** |

Conselho de Gestão do Patrimônio Genético
Situação cadastral conforme consulta ao SisGen em **12:52** de **10/11/2017**.

SISTEMA NACIONAL DE GESTÃO DO PATRIMÔNIO GENÉTICO E DO CONHECIMENTO TRADICIONAL ASSOCIADO - SISGEN

**Ministério do Meio Ambiente**
**CONSELHO DE GESTÃO DO PATRIMÔNIO GENÉTICO**

**SISTEMA NACIONAL DE GESTÃO DO PATRIMÔNIO GENÉTICO E DO CONHECIMENTO TRADICIONAL ASSOCIADO**

## Comprovante de Cadastro de Acesso

## Cadastro nº A5ACC65

A atividade de acesso ao Patrimônio Genético, nos termos abaixo resumida, foi cadastrada no SisGen, em atendimento ao previsto na Lei nº 13.123/2015 e seus regulamentos.

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|---|---|
| Número do cadastro: | **A5ACC65** |
| Usuário: | **Júlio Cezar Johner Flores** |
| CPF/CNPJ: | CONFIDENCIAL |
| Objeto do Acesso: | **Patrimônio Genético** |
| Finalidade do Acesso: | **Pesquisa** |

**Espécie**

**Caryocar brasiliense**

Título da Atividade: **Extração de polpa de pequi.**

**Equipe**

| | |
|---|---|
| **Júlio Cezar Johner Flores** | **UNICAMP** |
| **Maria Angela de Almeida Meireles Petenate** | **Unicamp** |

Data do Cadastro: **10/11/2017 14:47:18**

Situação do Cadastro: **Concluído**

Conselho de Gestão do Patrimônio Genético
Situação cadastral conforme consulta ao SisGen em **14:48** de **10/11/2017**.

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